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ARNOLD THEILER.

Director of Veterinary Research

(Union of South Africa).

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Contribution to the Study of Deficiency Disease, with special reference to the Lamziekte problem in South Africa.

By Sir ARNOLD THEILER, K.C.M.G. (Director of Veterinary Research),
HENRY HAMILTON GREEN (Biochemist, Veterinary Research
Division), and PHILIP RUDOLPH VILJOEN (Veterinary
Research Officer, Armoedsvlakte).

**From the Onderstepoort Laboratories for Veterinary Research,
Pretoria, March, 1915.**

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I. INTRODUCTION.

IN the *Second Report of the Director of Veterinary Research*¹ one of us (Theiler) reviewed at considerable length all the evidence available at the time of writing, concerning lamziekte, or gal-lamziekte, a disease affecting cattle in South Africa, and responsible for losses of stock so serious as to threaten to render cattle-raising in certain extensive areas of the country altogether impossible.

¹ Second Report of the Director of Veterinary Research. Department of Agriculture, Union of South Africa, 1912.

In the report mentioned the various theories as to the cause of lamziekte are discussed in relation to the evidence collected from the farming community of the affected areas, and in the light of the experimental work carried out by the staff of the Veterinary Research Division and other investigators.

The symptomatology and distribution of the disease need not be detailed again here, but the disease itself may be briefly stated as one primarily of the muscular system, and co-ordinated thereto, of the nervous system. Post-mortem findings are variable and mainly negative in character, no constant macroscopical lesions being found. Microscopically, fairly constant lesions have been recently established by Hedinger². In life, lamziekte, as the name implies, usually first manifests itself in a characteristic stiff or lame gait. A per-acute attack may appear suddenly and terminate fatally within a few days. A mild attack may last for days or weeks and either terminate fatally or in recovery. Recoveries are not infrequent, but a second attack usually ensues later, almost invariably with fatal results.

The superficially analogous disease of stijfziekte is not taken into consideration in this paper, since, though one of the forms of stijfziekte somewhat resembles lamziekte in its clinical aspects, there is no reason to believe that the two diseases, as such, or any of the forms of stijfziekte *inter se*, are of similar origin. It is possible that the different forms of stijfziekte are genetically different.

Of the various theories advanced from time to time to explain lamziekte, the "food deficiency" or "want of nutrition" hypothesis has perhaps maintained the longest and strongest hold throughout the country. Theiler (*l.c.* 1) discountenanced this view in the general sense, on the ground that it failed to explain all the observations in connection with the disease. At the same time many of the observations discordant with such a theory were not specifically experimental in character, while the view expressed by many farmers that the disease was less prevalent amongst cattle not exclusively confined to veld pasturage but receiving supplementary rations, left legitimate room for difference of opinion.

The progress, within recent years, of research upon diseases attributable to dietetic deficiency, more particularly the research upon the beriberi problem, naturally gave a fresh impetus to the deficiency theory for lamziekte and provided its adherents with a fresh working hypothesis whose validity could best be settled by direct experiment. Funk,³ in London, whose attention was drawn to the question by Dr. Donald Macaulay (Johannesburg), inclined to regard lamziekte as an avitaminosis of the beriberi type, while in South Africa the theory found a whole-hearted advocate in Mr. Stead, lecturer in the Grootfontein School of Agriculture.

In view of the attention which the vitamine hypothesis had attracted, and of the great general scientific interest of dietetic deficiency in itself, the experiments recorded in this paper were included as part of a larger scheme of work still in progress—many

² E. Hedinger, Pathological Investigation into Lamziekte. Department of Agriculture, South Africa, 1915.

³ C. Funk. "Die Vitamine, ihre Bedeutung für die Physiologie und Pathologie." Bergman, Wiesbaden, 1914.

of the animals experimented upon serving the dual purpose of testing the vitamine theory, and of providing material for investigations in other directions. The results of collateral investigations on the pathological-anatomical side are embodied in the report of Prof. E. Hedinger (*l.c.* 2) to which reference has already been made.

The existence of Casimir Funk's highly stimulating monograph (*l.c.* 3) renders preliminary discussion of the extensive literature on the avitaminosis question superfluous. In that monograph an exhaustive bibliography is given, and any reader unacquainted with its theme may be referred to it, both for an able discussion of the views of other workers and an instructive presentation of the author's own outlook. In place then of a needless survey of the literature, we may merely quote verbatim Funk's summary of his view concerning our own immediate problem (*l.c.* 3, p. 152):—

“Kurz zusammengefasst entsteht Lamziekte höchst wahrscheinlich durch Fütterung mit ausgetrocknetem Gras, verläuft akut oder subchronisch mit Paralysen, Kontrakturen und Gastroenteritis, ist durch Diätwechsel zu verhüten, ist nicht verimpfbar und besteht anatomisch in Herzerweiterung, Gastroenteritis, Ergüssen in serösen Häuten und Hämorrhagien. Obwohl mikroskopische Untersuchungen des zentralen und peripheren Nervensystems fehlen, ist die klinische und anatomische Ähnlichkeit der Lamziekte mit der Beriberi ganz frappant. Es ist deshalb eine Untersuchung der Frage in dieser Richtung notwendig.”

Concerning the pathological lesions here given by Funk and compiled from various reports made by different veterinary surgeons in South Africa at different times, comparison should be made with Hedinger's more recent report (*l.c.* 2), in which also an opinion is incidentally expressed on the avitaminosis aspect of lamziekte. In reality too much reliance cannot now be placed upon some, at least, of the sources from which Funk drew his conclusions, and in the present communication we propose to show, as main inference from our series of dietetic experiments, that lamziekte cannot be prevented or cured by a simple change of diet, and that in all probability the disease is *not dietetically* connected with the drought herbage which forms the staple feed of cattle in affected areas. Incidentally it may be mentioned that the so-called “sour grass” to which Funk draws special attention (*l.c.* 3, p. 151) merely means “coarse grass,” the distinction between “sour veld” and “sweet veld” being rather misleading and in some cases of geographical rather than dietetic significance.

II. SCHEME OF WORK.

The scheme of work to be considered in this communication comprises, briefly:—

(a) Rice-feeding experiments upon cattle, horses, sheep, goats, and pigs, with the object of producing, if possible, deficiency disease in the animals concerned, and of comparing the symptomatology of such disease with gal-lamziekte.

(b) Experiments with pigeons with the primary object of testing various rations used in the other experiments, in respect to their anti-neuritic efficiency or vitamine content.

(c) Experiments on dogs with the object of inducing an avitaminosis by rice-feeding, and of testing the effect of feeding flesh of cattle which had died from lamziekte.

(d) Feeding experiments upon cattle running on the lamziekte camps at the experimental farm, Armoedsvlakte, near Vryburg, Bechuanaland.

In this case the object was to determine whether or not lamziekte, in its natural incidence, could be (1) controlled prophylactically by the use of supplementary rations of known high vitamine content, (2) successfully treated with vitamine extracts, (3) increased by partially substituting polished rice, known to be deficient in vitamine, for the natural pasturage, (4) influenced by factors which affected the condition of the cattle without seriously affecting the vitamine content of their diet or the extent of natural grazing.

(e) Feeding experiments with hay cut at random from lamziekte camps, with the object of eliminating as far as possible all factors other than the purely dietetic.

(f) An attempt to co-relate the incidence of disease with the rainfall and condition of the veld on the area concerned, in respect to hypothetical vitamine deficiency of the natural pasturage.

III. LABORATORY EXPERIMENTS.

(1) *Pigeon Feeding Tests*.—The experiments with pigeons may be taken first, since they afford some indication of the character of the various foodstuffs used in the subsequent trials, in respect to their antineuritic value or content in beriberi vitamine.

The method adopted to determine the antineuritic value of the stuffs fed was that adopted by Gryn's, Schaumann, Cooper⁴, Funk, and others, in which the length of time elapsing between the onset of polyneuritis gallinarum (experimental beriberi) in birds fed on standard rations of polished rice, is compared with the time elapsing when known quantities of supplementary foods are added to the standard rice diet. Usually some definite experimental period (Cooper, 50 days) is selected, and the minimum amount of supplementary ration required to protect the birds from polyneuritis within this time is determined.

In the first preliminary series of our experiments the supplementary substance was ground, where necessary, made into pills with sugar, or into dough with a little boiled rice, and fed to each bird by hand, while raw rice was supplied *ad lib.* in the cages. Initially the pigeons all ate well over one-twentieth of their own body-weight of rice, but after a few weeks many of the birds ate so little of their own accord that forcible hand-feeding was resorted to in the majority of cases after about the fortieth day, the quantity of rice administered being equivalent to one-twentieth of the weight of the birds concerned.

In these, as in all other rice-feeding experiments on all animals, the best quality of table rice was used to ensure a highly polished grade. The bran, bean-meal, treacle, and linseed-oil were samples

⁴ Cooper. "On the protective and curative properties of certain foodstuffs against Polyneuritis induced in birds by a diet of polished rice." *Journal of Hygiene*, Vol. 12, p. 436, 1912.

of the material used in the Armoedsvlakte trials. The following Table I summarizes the results:—

Table I.

Bird No.	Diet.	Survival days.	Symptoms.	Bird No.	Diet.	Survival days.	Symptoms.
1	Rice alone	33	Polyneuritis.	23	Rice + $\frac{1}{2}$ grm. each of bean, maize, oil, treacle	40	Weakness ?
2		42	"				
3		40	"	24		64	"
4		37	"				
5		33	Doubtful.	25	Rice + 1 grm. hay	26	Polyneuritis.
6		36	Polyneuritis.			44	"
7	Rice + 1 grm. bean meal	80	Weakness ?	26			
8		62	Weakness.			27	Rice + 5 grms. hay
9	Rice + 5 grms. bean meal	85	Remained healthy.	28		32	Suffocation ?
10		85			29	Rice + $1\frac{1}{2}$ grm. yeast	85
11	Rice + 1 grm. maize	71	Weakness ?	30		85	
12		60	Polyneuritis.	31	Rice + 1 grm. fresh bones	54	Weakness ?
13	Rice + 5 grms. maize	85	Remained healthy.	32		46	Polyneuritis.
14		85			33	Rice + $2\frac{1}{2}$ grms. healthy biltong	46
15	Rice + 1 grm. treacle	53	Polyneuritis.	34		85	Healthy.
16		65	Weakness ?	35	Rice + 5 grms. healthy biltong	80	Polyneuritis.
17	Rice + 10 grms. treacle	18	Polyneuritis.	36		85	Healthy.
18		36	Enteritis.	37	Rice + 5 grms. lamziekte biltong (a)	75	Polyneuritis.
19	Rice + 1 grm. linseed oil	53	Polyneuritis.	38		80	Weakness ?
20		23	Doubtful.	39	Rice + 5 grms. lamziekte biltong (b)	60	Polyneuritis.
21	Rice + 10 grms. linseed oil	17	Enteritis.	40			
22		23	"			85	Healthy.

At the end of 85 days when the remaining birds were put out of the experiment, they all appeared comparatively healthy. All, however, had fallen off considerably in weight, including the two yeast birds which, in the early stages of feeding, had gained appreciably. In the latter stages most of the birds were noticed to be affected with lice, and this, together with the long confinement in small cages, probably accounts for the general lowering of condition. Doubtless many of the cases put down as "weakness" really represent polyneuritis in chronic form, but it was not always easy to clearly distinguish the symptoms from those of simple starvation, or starvation complicated by irrelevant complaints. In such cases the fall in weight was usually very marked, sometimes as high as 50 per cent., while at the same time the birds were capable of standing normally though showing great exhaustion when forced to run. Sometimes, also, worm infection occurred as a complicating factor. Only those cases, therefore, in which polyneuritis appeared in definite acute form are recorded as such.

The results of this preliminary experiment are curiously irregular, the individuality of the birds evidently playing an important rôle.

Thus bird 33 developed polyneuritis in 46 days, i.e. not much later than one of the control birds on rice alone, while bird 34, on the same diet, remained healthy throughout the 85 days of the experiment. Bird 35, on twice the quantity of the same biltong as bird 34, developed polyneuritis on the 80th day, although its fellow in the same cage was still vigorous when put out of the test. The birds fed on rice alone showed a longer survival than was expected, but this is probably due to the fact that forcible feeding was only resorted to in the later stages of the experiment.

The birds fed on treacle indicate that the antineuritic value of this substance is negligible, since the birds receiving 10 grammes succumbed in much shorter time than the controls. This, as also the longer survival of the birds on only 1 gramme of treacle in addition to rice, is probably due to the higher carbohydrate intake. The survival of bird 16 for 65 days is not easily explained, but in view of the irregularity of early intake nothing need be said. The linseed-oil tests were invalidated by enteritis on the larger intake, and showed no protection on the smaller. The ground bones, as expected, appeared to have little effect. This material was only included on account of its supposed value (in small doses) in preventing lamziekte. Hay, either in the small or the large quantity, had no effect. The fact that $1\frac{1}{2}$ gramme of pressed yeast was effective in preventing polyneuritis in this test, as against $2\frac{1}{2}$ grammes in Cooper's tests, is probably also due to the irregularity of rice intake during the earlier half of the experiment. In all subsequent tests a regular high intake was enforced by hand-feeding.

That the voluntary feeding of the birds results in a longer survival than usually maintains where a regular daily amount of rice is forcibly fed is incidentally indicated by the following records (Table II), which show the influence of varying treatment of the rice itself. The daily dry-weight of rice fed is the same in each case, and equivalent to one-twentieth of the body-weight of the pigeons—three birds in each test.

Table II.

	<i>Ad lib.</i> IN CAGE.	FORCIBLY FED BY HAND WITH RICE $\frac{1}{20}$ TH.			
	Raw Rice.	Boiled Rice.	Rice Parboiled.	Autoclaved Rice.	Washed Rice.
Polyneuritis devel- oped after.....	39 days	19 days	20 days	19 days	15 days
	33 "	20 "	25 "	19 "	24 "
	42 "	22 "	15 "	30 "	19 "

It is apparent that the difference between polished rice parboiled (i.e. ground and swollen into a dough with the minimum of hot water), rice boiled, rice autoclaved at 130° C., and rice washed for thirty-six hours in running water, lies within the limits of individuality of the birds. The vitamine content of the rice appears to be so low that the further reduction possibly incident to washing or autoclaving appears to make no practical difference. In the case,

however, when raw rice is eaten naturally at the discretion of the birds, the quantity ingested falls off very much after the first few weeks and the onset of polyneuritis is considerably delayed. Incidentally the observation of Abderhalden and Lampe⁵ that pigeons fed on cooked rice live longer than those fed on raw rice, does not seem to hold where truly equivalent weights of rice are fed.

The next summary (Table III) illustrates the influence of quantity of rice metabolised, upon the rapidity of onset of polyneuritis—rice ground, made into a dough with the minimum of boiling water, and forcibly fed.

Table III.

	Water Only (Starvation).	Rice Equivalent to Fraction of Body-weight.				
		$\frac{1}{30}$	$\frac{1}{40}$	$\frac{1}{50}$	$\frac{1}{60}$	$\frac{1}{70}$
Survival in days .	16	50	43	27	16	9
	14	46	29	22	16	15
	11	55	39	23	22	16

All the rice-fed birds developed acute polyneuritis, with the exception of the one surviving 55 days, which showed the chronic form.

Again the individuality of the birds is apparent, but at the same time it is fairly evident that the onset of polyneuritis is hastened by increase in the quantity of rice metabolised. Cooper (*l.c.* 4) and Funk⁶, and others, have made similar observations.

Whether the vitamines requirements of a bird are specifically related to its carbohydrate metabolism or, more generally, to its gross energy metabolism, it is difficult to say. From the results of other experiments, presently to be discussed, we believe the latter. As basis for all experiments hereafter recorded, equality in gross energy metabolism was aimed at, a starch-equivalent for the various supplementary rations being assumed and allowance made in deductions of rice, so that all diets correspond roughly in starch value to rice equivalent to one-twentieth of the body-weight of the birds. In any case, between the limits one-fifteenth to one-twenty-fifth, a few grammes of rice is unimportant in so far as survival-time is concerned. In all these experiments, also, feeding was forcibly carried out, so that except in occasional cases where a bird refused to empty its crop of the food of preceding days, the daily rations were always quantitatively ingested.

The results of tests with rice, bran, bean-meal, maize products, potatoes, coarse fodders, fresh meat, dried meat, normal biltong, lamziekte biltong, stijfziekte biltong, and vitamines extracts, may now be taken seriatim. It was not considered necessary to repeat the tests with treacle and linseed oil.

In all cases the ground supplementary stuff was mixed with ground rice, worked into a dough with the minimum of hot water, and so stuffed. The birds were usually three in a cage, and the dough for

⁵ Abderhalden & Lampe. "Zeitschrift für die gesamt. exper. Medizin," 1, 296 1913.

⁶ Funk. Proc. Physiol. Soc., Dec. 13, 1913, XXV.

all three was divided at sight according to the weights of the birds, which ranged from 270 to 330 grammes, the majority being about 310 grammes. The error of sight division is probably averaged out every three days or so, and in any case the weighing out and making up of each individual bird's ration would have involved an inordinate amount of time, since so large a number of pigeons had to be fed daily.

Rice Controls.—The survival periods of twenty-four pigeons developing polyneuritis on polished rice in the various tests made from time to time as controls ran: 22, 15, 15, 21, 16, 14, 22, 13, 13, 9, 14, 21, 21, 23, 17, 24, 16, 16, 22, 25, 28, 43, 25, 33 days respectively. Of these, twenty-two showed definite polyneuritis in acute form, while the two surviving 33 and 43 days, respectively, showed the chronic form. Our general experience is that the chronic form is much commoner amongst birds left to eat rice at their own discretion, while birds forcibly fed almost always develop the acute form. A probable reason for this is that the handling incident to artificial feeding tends to induce the acute form—the birds at any rate usually showed the first acute symptoms immediately after handling. The naturally fed birds, by voluntarily reducing their intake of rice, also probably stave off the rapid utilization of body vitamines reserves, so inducing a tendency for the disease to take the chronic form.

Taking the twenty-four birds as controls, we have an average survival of 21 days.

Bran, as used in the Armoedsvlakte experiments. Of three birds fed upon 1 gramme of bran, in addition to rice, one developed a fibrous tumour in the throat and had to be killed after 72 days' feeding. Another died on the 100th day, showing tapeworms on post-mortem. The third was put out of the test after 130 days, showing only a slight drop in weight of 5-10 per cent., beginning after the 85th day.

Of four birds, each fed on 2 grammes of bran in addition to rice, all were carried over 100 days, during which time they remained perfectly healthy, and, finally, showed body-weights within 5 per cent. of the initial figures.

These few tests suffice to show the extraordinarily high antineuritic value of this stock of bran, a point of very considerable interest in connection with the Armoedsvlakte experiments to be discussed later. The rice-fed birds acting as controls during the same period developed acute beriberi in from 16 to 22 days.

Bean-meal, as used at Armoedsvlakte. Of three birds fed upon 2 grammes of bean-meal per day as supplement to rice, one developed polyneuritis on the 56th day; the remaining two died without clear symptoms on the 70th day.

Of three birds fed upon 4 grammes of bean-meal, all three were carried over 80 days in a healthy condition, showing only a slight drop in weight.

We may therefore assume the minimal antineuritic quantity as about 3 grammes.

Maize Products.—The preliminary experiment had shown 5 grammes of whole maize as apparently adequate to protect against experimental beriberi, while 1 gramme was definitely inefficient. Owing to the importance of maize as the main article of diet of working natives in South Africa, experiments have been carried out on a series of maize

milling-products, but the results obtained are being repeated before publication as a separate article, and at this point we need only consider whole maize grain, and "samp," i.e. a table preparation consisting of maize corn rasped in grating machines until the external layers are completely removed, leaving a very clean white endosperm.

Of three birds, each receiving a supplement of 2 grammes of whole maize per day, one developed acute polyneuritis on the 19th day, one died without fall in weight, and therefore probably from extraneous causes, on the 18th day, and one survived 44 days, then dying of beriberi in chronic form.

Of three birds, each supplied with 4 grammes of maize per day, all three survived the experimental period of 60 days without mishap and with no serious drop in weight (10 per cent.). Three rice-fed birds, which had developed acute polyneuritis, were then put on to 5 grammes of whole maize per day with raw rice *ad lib.* in the cages. One was apparently too far gone to recover, but the other two were kept on this diet for three weeks, during which time they slowly picked up weight and vigour.

It is apparent therefore that 4 grammes contains sufficient vitamine for average ordinary requirements.

"*Samp.*"—Of three birds allowed to feed *ad lib.* on this commodity, one developed doubtful polyneuritis on the 24th day, the other two restricted their intake after the first few weeks and died on the 52nd and 80th day respectively—both of apparent starvation.

Of three birds *force-fed* with ground samp equivalent to one-twentieth body-weight, two developed typical polyneuritis after 23 days; one died over-night on the 13th day. There can be little doubt therefore that samp is a deficient diet in the same sense as polished rice, and that the vitamine of maize, like the vitamine of rice, is located in the external layers of the grain. In this connection the analyses of Funk⁷ are of interest.

Potatoes.—Of four birds, each fed on 14 grammes of minced raw potatoes, equivalent to 3½ grammes of dry matter, in addition to rice, all four remained perfectly healthy and without important change in weight for an experimental period of 65 days. Doubtless still smaller quantities would have been effective.

Coarse Fodders.—Attempts have been made to determine the antinueritic value of (a) market veld hay, (b) oat-straw, (c) dead grass, the growth of a previous season and cut from the veld after six months of practically rainless weather (the Pretoria winter), (d) Camp C hay, i.e. hay cut at random from a control lamziekte camp at Armoedsvlakte at a time when a heavy mortality prevailed amongst cattle on adjoining camps, (e) green lucerne-hay, (g) fan-dried green maize as usually fed to cattle in the fresh state, (h) ganna bosch, (i) *Crotolaria burkeana*. The Camp C hay, which represents at least a portion of the natural grazing of the Armoedsvlakte cattle, was still greenish in tint and yielded a greenish ether-extract. It was by no means "scorched."

It was found difficult to get clean results with birds given coarse fodders. Owing to the bulky nature of the fodder-rice dough, the crop was uncomfortably full after each daily feeding, and

⁷ Funk. "Maize and Pellagra." *Journal Physiol.*, XLVII, Dec., 1913.

many of the birds attempted, with more or less success, to regurgitate. In some cases, even when a bird appeared fairly healthy during the day, and more especially if it showed signs of general weakness, it died during the night, leaving diagnosis doubtful. In some of such cases it appeared as if death by suffocation had ensued as a result of clogging of the trachea in the attempt to regurgitate. It was not found feasible to feed more than 6 grammes of the coarse fodders per day, but the general results nevertheless show certain points of interest in connection with the cattle tests subsequently to be discussed.

(a) *Pretoria Veld-hay*.—Of four birds fed on 4 grammes, in addition to rice, two developed definite polyneuritis in 24 and 25 days respectively. The third died during the night of the 23rd day without however having shown any previous signs of polyneuritis or of weakness. Of ten birds fed on 6 grammes of hay as supplement to the rice ration, four showed definite polyneuritis after 20, 21, 30, and 43 days respectively. One survived 45 days and another 52 days, both dying either of polyneuritis in chronic form or of weakness, and showing a drop in weight of from 35-40 per cent. The remaining four died after 5, 16, 21, and 33 days respectively, the cause of death being uncertain, and perhaps due to overstuffing and subsequent regurgitation. The bird dying after 33 days had shown marked weakness and loss of weight during the preceding week and might have been on the verge of polyneuritis. The bird succumbing after 5 days died with a full distended crop.

From these results it is apparent that the antineuritic effect of the veld-hay is at least very low, although the comparatively long survival of three of the birds suggests that the 6-gramme quantity does display some slight protective effect. On the other hand this may be interpreted as individual high resistance of the birds themselves, and it is in any case unsafe to draw any conclusion from a survival period of less than 50 days.

(b) *Oat-straw*.—Two birds receiving 4 grammes of ground oat-straw both developed acute polyneuritis within 28 days. Of four birds receiving 6 grammes, one died on the 28th day after a few days of general weakness, one developed acute polyneuritis on the 28th day, another on the 37th day, while the fourth bird died overnight after 44 days, showing a fall in weight of about 30 per cent.

These tests may be taken as showing that oat-straw in quantities up to 6 grammes does not meet the deficiencies of twice its weight of rice.

(c) *Dead Grass*.—Of three pigeons fed with 6 grammes of ground material in addition to rice, two developed typical polyneuritis after 18 and 25 days respectively. The remaining one died on the 40th day, showing extreme weakness and a drop in weight of 45 per cent.

There can be little doubt that no protective influence was exerted.

(c) *Camp C Hay*.—Of four birds fed on 4 grammes each, one showed polyneuritis after 33 days, one had to be killed at the end of the first month owing to a fibrous growth developing in the throat, one died in a very weak condition after 52 days, while one survived 62 days, dying either of weakness or of chronic polyneuritis and showing a fall in weight of 44 per cent.

Of ten pigeons fed on 6 grammes as daily supplement to rice, three definite cases of polyneuritis occurred after 28, 31, and 35 days respectively. One died, presumably of suffocation on regurgitation,

after 4 days, another after 13 days, and a third after 28 days—this last probably being on the verge of polyneuritis. The remaining four all died overnight after 50, 62, 68, and 74 days respectively, showing weakness in varying degrees for a few days before death, but with no clear signs of polyneuritis, although a drop in weight of from 10 to 28 per cent. was recorded.

We think that these latter cases should not be wholly interpreted as idiosyncratic survival, and that they perhaps allow of the assumption that Camp C hay (immature) has slight antineuritic properties. The comparatively long survival of two of the birds on the 4-gramme quantity also supports this view, but on the other hand there is the fact that some of the birds succeeded from time to time in reducing their metabolic intake by partial regorgitation from the crop and hence lengthened their survival. In view of the tests with Pretoria veld-hay, in which the antineuritic effect is still more doubtful, we do not wish to be dogmatic about Camp C hay.

To test the effect of the mechanical nature of the hay-dough in itself, two trials were run in which 6 grammes of Camp C hay and 12 grammes of rice were fed daily along with an allowance of 0.1 c.c. of orypan (see later) in one case and 2-3 grammes of pressed yeast in the other. Of the orypan set, two birds were carried through 50 days without mishap. At the end of this time they were perfectly healthy, although showing a drop in weight of 5-15 per cent. The third died overnight on the 40th day for no apparent reason. Of the yeast set, one survived 50 days in excellent health and only slight fall in weight, while the other two died overnight after 20 days, presumably from suffocation in attempting regorgitation, or from damage in stuffing.

There is here an indication that in feeding mechanically unsuitable foods, it is unsafe to accept death as due to polyneuritis unless there is *very clear symptomatic evidence* for the assumption.

(f) *Green Lucerne-hay*.—Of four birds fed on 5 grammes of ground fan-dried lucerne as supplement to rice, one died within 24 hours and two more within three days. The fourth died after tolerating the lucerne for five days. The rapid and unaccountable death of these birds suggests an intoxication, but the point has not yet been specially investigated. Of four birds fed on 3 grammes of fan-dried lucerne, two also died on the fifth day. The quantity for the two remaining birds was therefore reduced to 2 grammes; one of these died after 45 days of feeding, but with no signs of polyneuritis and only a 10 per cent. drop in weight. The other was perfectly healthy after 65 days and showed no fall in weight.

It is fairly evident therefore that, although large quantities of lucerne are not tolerated, relatively small quantities suffice to protect against polyneuritis. We may safely assume that the vitamine content of green lucerne is high.

(g) *Crotolaria burkeana*.—The leaves of this bush were fed on account of its frequent distribution in certain areas upon which lamziekte and also one form of stijfziekte are found. Incidentally, it is the belief amongst certain farmers that crotolaria is capable of causing lamziekte, and in point of fact Theiler⁸ has shown that it is capable of occasioning a form of laminitis, which he designated as crotalism, when fed experimentally to cattle.

⁸ Theiler. "Stijfziekte in cattle." Union Agric. Journal, Feb., 1911.

Five grammes of dried green leaf were fed, along with the usual amount of rice, to three pigeons. At the end of the experimental period of 70 days all three were perfectly healthy and showed only a slight decrease in weight. One of these pigeons was then put on to rice alone and developed acute polyneuritis in 19 days, thus indicating that the lengthy survival on the bush is not to be interpreted as idiosyncratic.

Not only then has the green leaf of this bush no ill-effect on pigeons, but it has evidently well marked antineuritic properties.

(h) *Ganna-bosch (Salsola foetida)*.—Two trials were carried out with the leaves of this bush, since, according to the view of certain farmers, lamziekte is less prevalent on areas carrying ganna.

Three birds fed on 3 grammes of leaf, in addition to rice, all developed acute polyneuritis within 21 days. Of three birds fed on 6 grammes of the dried leaf, one died after 25 days, and another after 30 days, the cause of death being uncertain. The third survived for 50 days, showing marked weakness and a drop in weight of 30 per cent.

The antineuritic effect is therefore not high.

Flesh.—In the preliminary experiment no marked difference was discernible between biltong made from the flesh of healthy animals and that from the flesh of animals which had died of lamziekte. It was thought possible, however, that in the event of lamziekte being an avitaminosis, a depletion of tissue vitamine reserve might manifest itself in a lowered anti-neuritic value of the flesh, and this point was therefore tested in some detail. Stead, indeed, claimed to have detected a marked difference in this respect between lamziekte biltong and Karroo-grown flesh, but as a general discussion of Stead's experiments is given in Appendix II, they need not be specially considered at this point.

Normal Biltong.—In testing several different samples of normal biltong, or normal at least in the sense of "not lamziekte," it was found that considerable *inter se* variation occurred. In the following summary (Table IV), Samples I and II were ordinary butcher's biltong, Samples III and IV were prepared from cattle coming to the laboratory post-mortem table, III from a heifer dying of pericarditis as a result of accidentally swallowing a fragment of wire, IV from an ox killed because of a tumour in the spinal cord. Six grammes of biltong, equivalent to about 20 grammes of muscle, were fed in each case, as supplement to the rice ration:—

Table IV.

Bird No.	Sample I.	Sample II.	Sample III.	Sample IV.
1	Healthy after 60 days	Healthy after 60 days	Weak on 60th day	Polyneuritis 22nd day.
2	Healthy after 60 days	Polyneuritis 30th day	Polyneuritis 50th day	Polyneuritis 17th day.
3	Polyneuritis 45th day	Polyneuritis 29th day	Died 48th day...	Polyneuritis 22nd day.

With the first three samples it appears that 6 grammes of biltong, equivalent to about 20 grammes of flesh, was on the border-line of efficiency, while Sample IV appears markedly deficient. In this connection it is interesting to note that the onset of polyneuritis appears to incline to the "all or nothing principle."* A quantity of vitamine just below the individual metabolic requirements of a bird often seems only to delay the onset of beriberi for a short time (a few weeks), whereas a quantity just adequate protects indefinitely. With Sample IV above it is not safe to assume that because the birds survived no longer than they would probably have done on rice alone, the sample therefore contained little or no vitamine. We can merely assume that the 6-gramme quantity was inadequate. In connection with the death of bird 3, Sample III, the possibility of intoxication following upon putrefaction of the meat in the crop during three days in which the crop was not emptied in normal manner, must at least be considered. It might also, of course, be maintained that this is of little consequence since the failure to empty the crop may itself be an indication of incipient polyneuritis.

Lamziekte Biltong.—The following summary (Table V) shows a comparison between different samples of lamziekte biltong—three birds in each test:—

TABLE V.

Quantity of lamziekte biltong as supplement to rice.

Sample I.		Sample II.		Sample III.		Sample IV.	
5 grms.		5 grms.		6 grms.		6 grms.	8 grms.
46 days P		50 days ?		50 days ?		26 days P	30 days P.
21 „ P		115 „ H		50 „ H		26 „ P	36 „ P.
20 „ P		115 „ H		50 „ H		29 „ P	40 „ P.
Sample V.		Sample VI.		Sample VII.			
6 grms.	8 grms.	6 grms.	8 grms.	5 grms.	6 grms.		
35 days P	36 days ?	45 days P	22 days P	23 days P	32 days P.		
29 „ P	40 „ ?	24 „ ?	29 „ P	21 „ P	31 „ P.		
29 „ P	40 „ ?	29 „ P	50 „ W	13 „ P	27 „ P.		

* One of us (H. H. G.) is at present engaged in following out this point in detail, along with a number of other questions in connection with the finer details in the determination of the antineuritic value of food-stuffs. Meantime we do not wish to be dogmatic, since there are so many factors to be taken into consideration in dealing with the relation between vitamine requirements and survival time. The chief of these appears to be the rate of utilization of the body-reserves of the pigeon. Our present view is that a difference of one-fifth in the amount of vitamine ingested reduces the survival time from "infinity" (on the minimum adequate amount) down to about 37 days. Reduction by another fifth then only brings the time down to 21 days.

In this table definite polyneuritis is denoted by P, marked weakness by W, death from unknown causes by an interrogation mark. Where the birds were put out of experiment in healthy condition the letter H is used. The two birds which remained healthy over 115 days on Sample II were then roughly tested for individual resistance by being put on to rice alone. Both developed polyneuritis in 16 days, just as they might have done without previous experimental diet. Cooper (*l.c.* 4) regards 5 grammes of air-dried healthy flesh as sufficient to protect birds (receiving one-twentieth body-weight of rice) for over 50 days. In most of these cases, therefore, the samples of lamziekte biltong showed a markedly lower antineuritic value than that regarded by Cooper as normal. On the other hand, one of the biltongs (IV) in Table IV, normal in the sense of "not derived from a lamziekte beast," showed an equally low anti-neuritic efficiency, while lamziekte Sample II, Table V, compares favourably with normal flesh.

Table V does *not*, therefore, allow of the deduction that lamziekte flesh is deficient *in virtue of* its origin from lamziekte beasts.

Stijfziekte Biltong.—Two samples of biltong from cattle suffering from stijfziekte in the "hoof form" (*l.c.* 2) were tested—stijfziekte being a disease for which the deficiency theory has also been advanced. Of three birds on 5 grammes of the first sample, two succumbed to polyneuritis after 17 and 38 days respectively, while one died on the 18th day for some unknown reason but without appreciable fall in weight. Three birds on 8 grammes of this sample (plus rice as usual to bring up the calorific value to the one-twentieth rice equivalent) all remained healthy for an experimental period of 50 days, although all decreased somewhat in weight.

Of three birds fed on 5 grammes of the second sample all developed polyneuritis within 25 days. Three birds on 8 grammes showed polyneuritis after 25, 35, and 38 days, respectively. Since even 8 grammes offered little or no protection it was considered of interest to try the effect of feeding this biltong alone. For comparative purposes a sample of normal biltong, and a mixed sample of lamziekte biltong were also fed as sole diet. Ten grammes were given in each case to duplicate pigeons. Of the two birds on stijfziekte biltong alone, one developed typical polyneuritis on the 26th day, the other died in a very weak condition on the 28th day, showing a drop in weight of 33 per cent. but no acute polyneuritic symptoms. The two birds on normal biltong alone were quite healthy and showed no serious (5-10 per cent.) change in weight when put out of experiment after 70 days. The two birds on lamziekte biltong were carried on for 118 days, at the end of which time they were quite healthy, one retaining its weight throughout except for minor fluctuations, the other falling off somewhat in condition and showing a final drop in weight of 10-15 per cent.

Evidently 10 grammes of normal or lamziekte dried flesh was sufficient to cover the dietetic requirements of the pigeons in all vital respects. The stijfziekte biltong, however, shows an antineuritic value, or vitamine content, lower than that required in the metabolism of the material itself. This is of particular interest as raising two points, (a) the relation of vitamine deficiency to specific carbohydrate metabolism, and (b) the question as to whether the observed deficiency was one of the original flesh or one arising in the process of making the biltong. In regard to (a), there is evidence that the onset of

beriberi is related to the *total* exogenous metabolism and not to the exogenous carbohydrate metabolism. In the experiment under discussion, where the total calorific value of the dried meat (with about 8 per cent. of fat) is distributed roughly four-fifths from protein and one-fifth from fat, we have a case of clear polyneuritis occurring on a diet free from carbohydrate as such. It must, of course, be kept in mind that over 40 per cent. of flesh protein may, according to Lusk, be theoretically converted into glucose in the process of katabolism, and that therefore in the narrower sense the relation between vitamine consumption and carbohydrate metabolism may still be a specific one. Since it is not feasible to exclude glucose metabolism within the cells by any system of dietetics the question does not offer hope of direct solution. Funk and Schönborn⁹ have, however, adduced evidence suggesting a tendency towards hyperglycaemia in pigeons on vitamine-free diets, and, should this prove to be the rule, the fact would argue in favour of a specific connection between carbohydrate metabolism and vitamine requirements. In Funk and Schönborn's experiments, however, the hyperglycaemia is not noticeable on the "fat-free" diet and in the single pigeon on this diet which also received vitamine, the percentage of blood sugar is higher than in those receiving no vitamine. On the "sugar-free" diet, where the total calorific equivalent is distributed in the approximate ratio, casein, starch, fat: 1 : 3 : 7, the hyperglycaemia is most marked. At the same time the only bird which chanced to develop polyneuritis within the 14-day experimental period was fed on this diet of preponderating fat content.

On the whole it seems reasonable to assume that the quantitative vitamine requirements of an organism are determined by the gross metabolism of the cells irrespective of the nature of the vitamine-free stuff metabolised.

In relation to point (b), it was considered possible that a falling off in antineuritic efficiency might take place in the drying-out process of biltong making. A quantity of healthy lean beef was therefore cut into strips and divided into three. One part was converted into biltong in the shade, one part in the open, while the third portion was minced, fan-dried, and stored. These three samples were then fed to pigeons in triplicate with the results indicated in Table VI.

Table VI.

Rice as usual, with material fed in quantities of—

	Fan-dried.		Shade-dried.		Sun-dried.		Rice Controls.
	6 gms.	8 gms.	6 gms.	8 gms.	6 gms.	8 gms.	1/20th body-wt.
Days ensuing before onset of polyneuritis.....	21	29	23	39	27	43	22
	22	45	33	30	13	27	13
	21	65	30	35	13	63	13

No constant difference is noticeable between the three samples of material, and from the fact that even the 8-gramme quantity

⁹ Funk and Schönborn. "The influence of a vitamine free diet on the carbohydrate metabolism." *Journal Physiol.*, XLVIII, No. 4, p. 328, 1914.

was not always adequate to protect against polyneuritis over Cooper's period of 50 days, it would rather seem that the original sample of beef was of unusually low anti-neuritic value. The experiment as such was not repeated, but two samples of lamziekte biltong from the same animal (Armoedsvlakte) were procured, one shade-dried and the other sun-dried. Fed in 8-gramme quantities to triplicate pigeons both samples protected efficiently for 50 days.

To note whether simple drying affected the antineuritic value of fresh meat a quantity of butcher beef was obtained, minced, fan-dried, ground, and bottled. A month later this was fed in 6-gramme quantity with rice as usual. At the same time fresh meat was procured every second day, minced, and fed in 20-gramme quantity raw and in 5 and 8 gramme quantity fan-dried. The results are indicated in Table VII.

Table VII.

Fresh meat *v.* dried meat. Rice as usual:—

20 gms. Fresh Meat.	5 gms. Fan-dried.	8 gms. Fan-dried.	6 gms. Fan-dried Stored.
1. Died overnight 37th day.	Died overnight 35th day.	All fairly healthy on 50th day	Polyneuritis 18th day.
2. Rather weak on 50th day.	Polyneuritis 21st day.		Polyneuritis 18th day.
3. Quite healthy on 50th day.	Polyneuritis 19th day		Polyneuritis 21st day.

The fresh meat appeared to be more nauseating than the dried meat. Pigeons 1 and 2 on fresh meat often attempted to regurgitate from the crop, and it is not improbable that bird 1 was suffocated in the process. Bird 2 systematically regurgitated about half its food. Bird 3 retained its food well, and after fifty days showed very little change in weight. From the table it appears that 5 to 6 grammes of the fan-dried meat, equivalent to about 20 grammes of the fresh meat, was not sufficient to protect as effectively as the fresh meat itself. The 8-gramme quantity, corresponding to about 30 grammes of fresh meat, was, however, effective. This difference is not much to go upon, but there is at least the suggestion that some loss in antineuritic efficiency occurs during drying out—sufficient to bring a minimal protective quantity of flesh below the adequately protective limit. The experiment has not yet been repeated or extended, and it is admittedly not conclusive, but such as it is it at least serves to show that a sample of dried flesh cannot be regarded as abnormally low in vitamine even if quantities of less than 8 grammes do not protect pigeons from polyneuritis—a point of importance in relation to Stead's experiments (Appendix II).

Surveying all the data concerning meat and biltong feeding, and assuming that the majority of observed differences cannot be wholly explained on the basis of individual resistance of the different birds, it would appear (*a*) that normal flesh derived from different animals may vary considerably in antineuritic efficiency, (*b*) that no certain constant difference is detectable between normal biltong and lamziekte

biltong, (c) that in the process of preparation of biltong, and subsequent storage, some lowering of vitamine content may perhaps occur, (d) that voluntary muscle has a low antineuritic value.

Incidentally a few tests were made comparing liver, heart muscle, and voluntary muscle, and we are able to confirm Cooper's¹⁰ observation that cardiac muscle and liver have a higher antineuritic value than voluntary muscle, although in our experiments the differences observed were not nearly so marked as in Cooper's. We could not, however, observe the marked curative effect of dried liver powder, of which 7 grammes sufficed in Cooper's experiments to effect a cure lasting a week. In the four cases tried by us this quantity of dried healthy liver tissue only sufficed to effect a temporary improvement, and on resuming artificial feeding with rice all four birds developed a second attack of polyneuritis in from 24 to 48 hours. We have not made any extensive comparison of different samples of liver, and it is, of course, just possible that the vitamine content of this organ may be as variable as its fat content.

Two other tests of some interest have yet to be mentioned:—

(a) A quantity of ground lamziekte biltong was extracted with alcohol and precipitated with ether in the manner described by Cooper, and an amount of extract equivalent to about 1 lb. of the meat was mixed with a little rice and fed in two successive daily meals to a pigeon suffering from acute polyneuritis. The resulting cure further establishes, in qualitative fashion, the presence of vitamine in lamziekte muscle.

(b) The flesh of a number of pigeons succumbing to acute polyneuritis was fan-dried as collected. The amount obtained was hardly adequate for a prolonged feeding test, but a pigeon suffering from polyneuritis (in fair condition, however) was fed upon 5 grammes the first day, and 10 grammes on four succeeding days. Recovery ensued. Put on to 10 grammes of rice in addition to 10 grammes of dried polyneuritic flesh, it developed a second attack of polyneuritis five days later. This may be tentatively accepted as evidence that although the development of polyneuritis probably involves diminution in the vitamine reserves of the tissues, the depletion process is far from complete. The observation is also in accord with one of Funk's recent experiments, in which a curative extract was prepared from polyneuritic pigeon tissue.

GENERAL OBSERVATIONS ON THE PIGEON-FEEDING TESTS.

(a) *Limitations of the Test.*—It appears to us that the individuality of different birds represents a factor which is often underrated in experimental work on pigeons, and that there is a tendency to regard the determination of the antineuritic value of foodstuffs on a quantitative basis as easier than it really is. Conclusions drawn from tests on a small number of pigeons are naturally open to criticism, and in many of our own experiments we feel that the conclusions drawn can only be tentative. At the same time the necessary hand-stuffing of birds in batches, say, of fifty per test, instead of in sets of three to six, would involve an enormous amount of labour, and we believe that the nature of our tests, though admittedly limited, is sufficient for the purpose in view.

¹⁰ *L.c.* 4, also Part II, *Journ. of Hyg.*, 1914, Vol. 14, No. 1, 16.

When dealing with pigeons forcibly fed on moderately large quantities of rice alone, the results are clear-cut, and in nearly all cases the birds develop typical polyneuritis within four weeks. Here, however, the pigeons receive a diet of vitamine-content so far below the requirements involved in the high metabolism compelled by the quantity of food force-fed, that the onset of polyneuritis tends to occur in minimum time. In this connection it is interesting to again note the recognized fact that when the birds are allowed to regulate their own intake of rice, a large proportion of them successfully strike a balance which allows of survival for a period twice as long as that which may be regarded as a normal period for death either by beriberi or by simple starvation, and even then go under rather with the symptoms of delayed inanition than those of polyneuritis.

In the case where a high intake of food is ensured by artificial feeding, but at the same time supplementary antineuritic stuffs are included, the individual resistance of the bird again has a fair field. We have noticed for instance, that for one bird the vitamine supplied by 2 grammes of maize per day may suffice for long periods, while for another 4 grammes is necessary, with perhaps 5 or 6 grammes as a quantity in excess of *any* individual requirement. This individuality factor appears particularly marked when supplementary stuffs of low antineuritic value are used, and still more marked where the nature of the stuff offers scope for differences arising out of the different digestive capacities of different birds. Thus, for instance, in our experiments with Camp C hay, three of the birds developed polyneuritis in comparatively short time (28, 31, 35 days), while four survived for much longer periods (50, 62, 68, 74 days) without definite polyneuritic symptoms. It seems not unreasonable to assume that the hay did possess distinct antineuritic properties, but that those birds developing polyneuritis had failed to adequately utilize the vitamine supply owing to an incapacity to deal with the bulk of the fibrous material fed. That the vitamine theoretically at the disposal of the bird is not *wholly* available is suggested by Cooper's experiments (*l.c.* 10) with alcoholic extracts of the *excreta* of hens fed on unpolished grain, and rabbits fed on bread and cabbage. A series of Cooper's tests on milk is also of considerable interest, because we would expect the vitamine of milk to be more or less completely utilized. Vedder and Clark¹¹ had previously noted that of four fowls fed on polished rice plus 5 c.c. of cow's milk daily, two developed polyneuritis in a short time, while the other two remained healthy even after two months. The results of Cooper's milk-feeding tests may be summarized thus:—

Fresh Milk given	3 c.c.	4 c.c.	10 c.c.	12 c.c.	20 c.c.	20 c.c.	30 c.c.	35 c.c.	35 c.c.
Polyneuritis developed	18th	11th	11th	18th	33rd	36th	19th	49th	54th day
Fresh Milk given.....	2 c.c., 6 c.c., 15 c.c.								
Symptoms of weakness on.....	38th, 38th, 16th day.								

Here, although 2 c.c. and 20 c.c. appear to offer temporary protection, 3 c.c. and 30 c.c. offer no protection at all. The bird receiving 2 c.c. survived at least 38 days, while the bird receiving 30 c.c., or fifteen times as much, succumbed in 19 days. Admitting that such extremes are very rarely shown, the results forcibly illustrate the

¹¹ Vedder and Clark, 1912. "A Study in Polyneuritis Gallinarum." Phil. Journ. of Science, VIIb.

factor of individuality. They are also interesting in another sense, since although Cooper's conclusion that milk possesses only feeble antineuritic properties is perfectly justified in respect to pigeons, milk is known to contain ample vitamine for the health and growth of the human infant (beriberi subject) and the young of animals, during the very period in which metabolism is most active. Probably the *dry matter* of milk has a considerably higher antineuritic value than whole maize grain.

A foodstuff, therefore, which, tested in the usual way on the easily reacting pigeon, shows a low antineuritic value, may nevertheless prove itself adequate when fed to other animals. Where, for example, as in the case of veld hay or dead grass, it was found that the maximum quantity which could be successfully fed to pigeons was insufficient to offer any marked protection against polyneuritis, it cannot be assumed that the vitamine content is necessarily below the requirements of animals utilizing poor quality hay as their natural food. This point will be referred to later in connection with the cattle-feeding experiments.

(b) *Weekly Weight Records of Pigeons*.—In all cases in which definite polyneuritis occurred there was a fall in weight ranging from 12 per cent. to 50 per cent., with an average of about 30 per cent. In general, the longer the survival, the more marked was the fall in weight. We agree with Funk that the drop in weight is practically invariable when precautions are taken to empty the crop before weighing a dead bird. There may, however, be a minor fall in weight which is not associated with the vitamine content of the diet but due probably to a general falling off in condition as a result of the change from spacious store-room to confined experimental cage, and to the discomfort of artificial stuffing with dough-like foods. This is most marked with fully grown birds in fat condition. With birds in initially poor condition there may naturally be an increase. When the birds are weighed alive the weight of food present in the crop can only be guessed, and even with practice a margin of error of 5 per cent. of the body-weight is to be considered probable.

(c) *Orypan Treatment*.—Through the good offices of Prof. E. Hedinger, a considerable quantity of orypan, a vitamine preparation from rice-polishings, turned out by the *Gesellschaft für Chemische Industrie* in Basle, was placed at our disposal by that firm. This preparation was tested on pigeons to determine the curative dose and to obtain some basis upon which to proceed in testing the therapeutic value in lamziekte. It was found to be highly curative. In nine cases out of ten, doses of 0.2 c.c. diluted to 0.5-1 c.c., and injected subcutaneously, effected the cure of acute polyneuritis within a few hours. In a few cases 0.1 c.c. sufficed, while in a few others a second dose of 0.2 c.c. was required. In practically all cases where the birds were caught early in an acute attack, recovery could be readily brought about by orypan. Expressed on the weight of the birds the curative dose may be put down as 0.3-0.8 gramme per kilo. The minimum dose *per os* could not be so easily determined, but seemed to be rather higher. In relation to the curative dose it is interesting to note that although so small a quantity is curative, a somewhat larger total quantity seemed to be required to prevent the onset of polyneuritis, when the amount given was spread out in daily rations. Thus 0.02 c.c. of orypan added daily to a rice diet effected no appreciable delay in the onset of beriberi, although 0.2 c.c. sub-

cutaneously injected cured one of the very birds going down after 20 days on 0.02 c.c. per day. 0.05 c.c. daily seemed sufficient to protect indefinitely. The observation was made on three birds in each case, but has not since been repeated. After cure with orypan several birds were fed on rice again. All developed a second attack in from 3 to 9 days. If birds had survived for lengthier periods on diets of low vitamine content, before developing an attack, cure seemed to be more difficult, although a larger dose of orypan was even then usually effective, provided the symptoms of polyneuritis were quite clear. Sometimes if the birds were very weak at the time of attack and showed a very marked fall in weight, the acute symptoms were made to disappear, but nevertheless death followed through weakness.

(d) *Spontaneous Recovery*.—In connection with lamziekte it had been observed that the injection of certain pyrogenic substances (vaccine of anthrax, black-quarter, redwater, *B. coli*, etc.) was apparently of prophylactic value, and had even apparently effected a temporary recovery in certain beasts responding with a marked temperature reaction. It was considered of interest to try the effect of inducing a temperature reaction in polyneuritic pigeons, and accordingly three birds in the early stage of acute polyneuritis were injected subcutaneously with a 0.3 c.c. of an attenuated culture of a coli organism, 0.3 c.c. of yeast emulsion, and 0.3 c.c. of water, respectively. All three showed marked improvement, and on the following day had apparently recovered (perching normally). This rather astonishing result could not be attributed to a stimulus in utilizing ultimate vitamine reserves as the result of a temperature reaction, because the bird injected with water only, also recovered. A number of birds were then carefully watched, and rice feeding stopped the moment any symptoms of incipient polyneuritis were observed. Of these, four cases of polyneuritis occurring together were isolated and allowed drinking water only. Two (crops fairly full) died within twenty-four hours, the other two (crops nearly empty) apparently recovered, and, although weak, showed no typical symptoms of polyneuritis forty-eight hours afterwards. Of these two, one was allowed maize *ad lib.*, from which it ate gradually increasing quantities and steadily returned to comparatively normal health and weight. The other was again force-fed with rice and developed a second attack of polyneuritis on the following day. These observations were repeated, and quite a number of instances of this apparent momentary "starvation recovery" were noted, more particularly when the first attack occurred early in an experiment and on a comparatively empty crop.

In view of the possibility of such spontaneous recovery it may perhaps be questioned whether some of those cases of temporary improvement and short survival noted by Funk in his extremely interesting treatment of polyneuritic pigeons with compounds containing purin and pyrimidine groupings were wholly due to the substance administered, or whether they may not, in part at least, have possibly been spontaneous. The same applies to certain earlier experiments of Eykman, who claimed that the subcutaneous injection of small quantities of the mixed chlorides of sodium and potassium effected a cure in polyneuritic pigeons, and to those experiments where, in general, any conclusion is drawn from an observed improvement some hours after administration of an extract of doubtful antineuritic efficiency.

We may now turn to the experiments on mammals, but before doing so, brief mention may be made of a few experiments on fowls. Six fowls fed *ad lib.* on raw rice at first ate as much as one-tenth of their weight daily, but the quantity rapidly fell off until, at the end of 40 days, not more than one-hundredth was being taken. Forced thereafter with one-twentieth of their weight of rice, polyneuritis rapidly developed, 2, 2, 5, 6, 8, and 12 days later, respectively. Two birds, artificially fed from the beginning, both developed polyneuritis within thirty days. As in the case of the pigeons the difference between the effect of naturally regulated intake and forced metabolism at a moderately high level is well brought out. The curative dose of orypan (subcutaneous) was also tested on polyneuritic fowls and found to be about half a gram per kilo body-weight.

2. RICE-FEEDING EXPERIMENTS ON CATTLE.

The experiments on cattle are the most interesting of the laboratory set, since, owing to their importance in relation to the lamziekte problem, more detailed records of individual diet were kept than in the case of the tests on sheep and goats, to be subsequently discussed.

In the following Table VIII the weekly records of live-weight and of diet are given. Six cattle were experimented upon, young bull 3108, grown ox 1928, young heifer 2569, grown calf 2758, cow in calf 3024, cow in calf 3039. Each animal was separately chained up in the experimental stall during feeding to allow of controlling its intake. All animals were exercised by allowing free run in a fenced kraal covered with cinders but absolutely devoid of growing vegetation. In the intervals between feeding, during which the cattle were in the open, the stalls were barred to prevent any animal wandering back to the wrong manger and so vitiating the diet records. It was found impossible to feed rice alone, and a very small quantity of coarse fodder had therefore to be given for the sake of minimizing digestive disturbance. This varied from $\frac{1}{2}$ lb. to 1 lb. of poor quality veld hay per day, from October to the end of April, after which about 2 lb. of oat-straw autoclaved for two to three hours at 125° to 130° C. was supplied, except in the case of grown calf 2758, with which 1 lb. was found sufficient, and cow 3024, with which in the last few months of the experiment from 4 lb. to 6 lb. was found necessary to keep down the digestive disturbance. In autoclaving the straw 130° C. was aimed at, but owing to variable steam-pressure this temperature was not always maintained. After autoclaving, a slight increase in weight, ranging around 10 per cent., took place owing to condensation of steam within the straw, but this is allowed for in the record table. The polished rice was weighed dry, soaked in water overnight, and fed in quantity rather more than was expected to be eaten. Allowance was then made for the residue uneaten.

The data of Table VIII may, therefore, be taken as representing the actual quantities of stuff ingested to within an estimated error of about 10 per cent., although as a matter of fact the main results would not be vitally affected if twice this error were admitted. Cow 3039 is excluded from the table, but is considered separately along with the others in the text below. A transition week in which increasing quantities of rice were substituted for the ordinary diet served to bring the cattle on to the unaccustomed food. Salt was allowed in adequate amount. The experiments extended from 7th October, 1913, to 7th October, 1914, after which they had to be abandoned owing to the necessity for rearrangement in departmental work.

Rice Feeding Experiments on Cattle, showing weekly records of live-weight in kilograms, and of food eaten in lb.

DATE.	Successive Weekly Periods.	YOUNG BULL 3408.			GROWN OX 1928.			YOUNG HEIFER 2569.			CALF 2758.			COW IN CALF 3024.		
		Weight, in kilos.	Food eaten, in lb.		Weight, in kilos.	Food eaten, in lb.		Weight, in kilos.	Food eaten, in lb.		Weight, in kilos.	Food eaten, in lb.		Weight, in kilos.	Food eaten, in lb.	
			Rice.	Hay.		Rice.	Hay.		Rice.	Hay.		Rice.	Hay.		Rice.	Hay.
1913. October to October	7th.....	280	—	—	427	—	—	335	—	—	100	—	—	469	—	—
	14th.....	255	52	ad lib.	445	60	ad lib.	319	56	ad lib.	85	28	ad lib.	435	56	ad lib.
	21st.....	250	96	15	450	104	21	300	104	18	88	19	6	440	104	18
	28th.....	262	88	21	470	104	21	312	88	21	91	22	7	430	104	21
	November 4th.....	255	112	21	450	94	21	306	96	21	92	26	7	418	86	21
November	11th.....	254	116	21	475	112	21	310	112	21	90	17	5	415	115	21
	18th.....	267	112	14	467	112	17	303	111	17	86	21	7	417	110	17
	25th.....	262	112	14	465	112	14	308	112	14	90	24	7	430	112	14
	December 2nd.....	288	112	7	500	112	12	338	112	12	96	28	7	455	112	12
	9th.....	275	112	7	481	112	7	311	112	7	98	27	3½	431	112	7
December	16th.....	275	112	7	470	112	7	305	112	7	100	27	3½	410	112	7
	23rd.....	285	112	7	500	116	7	349	116	7	102	29	3½	432	116	7
	30th.....	270	126	7	480	126	7	321	126	7	91	35	3½	395	126	7
	1914. January 6th.....	290	110	7	490	118	7	325	121	7	100	26	3½	334	87	6
	13th.....	285	126	7	494	126	7	325	124	7	105	33	3½	338	126	7
February	20th.....	265	122	7	485	126	7	311	119	7	100	35	3½	310	109	7
	27th.....	265	122	7	480	124	7	300	126	7	90	33	3½	292	108	7
	3rd.....	275	113	5½	445	121	6	310	119	6	99	33	3	284	104	6
	10th.....	270	124	3½	450	126	3½	309	126	3½	103	35	1½	280	124	3½
	17th.....	264	124	3½	457	122	3½	310	126	3½	100	33	1½	280	121	3½
March	24th.....	252	122	3½	446	121	3½	310	126	3½	106	35	1½	275	120	3½
	3rd.....	290	117	8½	—	123	3½	299	125	3½	—	34	1½	250	120	3½
	10th.....	250	124	8	457	123	3½	311	126	3½	103	35	1½	270	119	3½
	17th.....	244	114	4½	—	123	3½	302	122	3½	102	35	1½	270	109	3½
	24th.....	255	126	7	472	124	3½	305	126	3½	105	35	1½	269	120	3½
April	31st.....	255	123	7	458	126	3½	305	125	3½	110	28	1½	262	121	6½
	7th.....	250	126	7	437	124	3½	300	126	3½	105	30	1½	265	122	7
	14th.....	—	118	9	—	111	3½	—	119	3½	—	31	1½	—	101	9
	21st.....	255	109	6	425	102	4	305	105	3½	110	30	1½	277	117	11
	28th.....	265	100	10	417	100	11	310	106	10½	112	29	1½	267	84	14

Before summarizing Table VIII in the form of totals each animal may be discussed somewhat in detail.

Young Bull 3108.—Nothing worthy of special comment was noted until about 17th February when a bad attack of diarrhœa set in. This partially abated, but returned again on the 24th. Further attacks occurred about 11th March, 18th April, 25th June, and 10th August, with minor digestive disturbance in between.

It finally died on 24th September showing post-mortem lesions of gastro-intestinal catarrh and slight hydropericardium. There was but little food in the stomach at death, and the organs showed a paler colour than usual, probably indicative of anaemia. During the survival period of almost exactly a year upon the diet of rice and extremely small moiety of coarse fodder, the body-weight fluctuated around the original figure of 280 kilos, falling as low as 220 kilos at the beginning of March after the early digestive disorder, but rising again later to above the original weight. A week before death the weight was practically the same as at the beginning of the experiment. On a normal diet, of course, this young bull would have gained considerably in weight.

Throughout the whole year no symptoms resembling those of lamiektie, nor any which could be justly diagnosed as the manifestation of a specific deficiency disease, could be observed. About 27th June a swelling of the near hind fetlock and a consequent lameness, more noticeable when the weight came on the foot, were noted. This may have been due to mechanical injury, or may have been a laminitis similar to that observed in the ox next to be discussed. The skin of the animal sometimes showed an unhealthy appearance with superficial roughness or scurf on the neck and dewlap, but as this occurred quite early in the experiment and was not progressive in character no importance can be attached to it.

Ox 1928 showed slight digestive disorder from time to time with a bad attack of diarrhœa early in April. This persisted, and on 30th April a little blood was passed with the faeces.

Considerable improvement took place in the ensuing weeks, but at the end of May another attack occurred, the animal being noticed unwell, and lying down while the other cattle were standing. When forced to move, however, it got up easily and stretched itself like a healthy beast. On the following day it was decidedly unwell and only ate 4 lb. of rice. Hanging of the ears, slight dribbling from the mouth, and grinding of the teeth were observed. When standing, the off hind leg was occasionally raised to ease the weight off the foot, and when urged to walk the animal showed lameness in this leg, thrusting it further forward than the right leg, and easing the weight off it as quickly as possible as if in pain. A pronounced quick, short gait resulted, but the animal did not show the same tendency to lie down as on the previous day. Thereafter considerable improvement took place, especially after treatment with bismuth subnitrate, but a stiffness in the fore legs persisted for some days. On 27th June still more noticeable symptoms were shown. The animal ate very little rice and remained lying down the greater part of the day. When disturbed it rose with great difficulty, lifting the hindquarters, remaining on the knees for some time, alternating the position of the hind feet, and then struggling to the standing posture. It walked with short, stiff painful steps, kept grinding the teeth, and showed

greenish sour diarrhœa. These almost alarming symptoms abated however, and two or three days later the beast could walk fairly easily, with normal stride, although with some irregularity of gait and slight pain in the feet.

On 18th July and 4th August much milder manifestation of the same symptoms appeared, but thereafter the animal settled down to a fair state of general health which continued until 13th October, when it was put out of experiment after a full year on the rice diet. From an initial weight of 427 kilos it finally fell to 385 kilos, although so late as 25th August it registered its original weight. This ultimate drop of 10 per cent. cannot be regarded as having any significance. Indeed, it is surprising that the ox maintained its general health so well in spite of persistent periodic diarrhœa. When finally shot for post-mortem, a slight duodenal cattarrh was noticed, and a general paleness of the organs suggestive of anaemia.

The symptoms shown during life could not be said to resemble lamziekte nor to justify diagnosis of anything more than periodic indigestion associated with a recurrent dietetic laminitis.

Young Heifer 2569 showed pronounced stiffness with swollen fetlocks towards the end of December and walked with an awkward laminitic gait, not, however, to be confused with "lamziekte gait."

This passed off naturally, and except for slight signs of digestive disturbance no other symptoms were noticed right up to the time when the animal was discharged from the experiment after the expiry of a full calendar year from commencement of rice-feeding. The body-weight had fluctuated to some extent, but finally showed an increase of 40 kilos, or 10 per cent., above the original figure.

A few days after being put out of experiment the heifer calved, having evidently mated unobserved with the young bull three months after rice-feeding was begun. The calf curiously enough was born blind, the blindness being apparently dependent upon some alteration in the optic nerves or their endings (amaurosis), since the eye itself appeared normal.

Calf 2758 (eleven months old bull) developed diarrhœa on 6th November, one month after the experiment started. On 10th November a little blood was passed with the faeces. This diarrhœa passed off and nothing worthy of special comment was noted until 2nd June, when a lameness in the off fore leg became apparent. Shortly after this the calf suffered accidental fracture of the left femur and had to be separated from the other animals in the kraal. Rice-feeding was, however, continued to 11th August, but the bone fracture did not heal well, and the calf, which was very troublesome to feed in the recumbent position, was put out of discomfort. Post-mortem showed no characteristic lesions, though perhaps slight anaemia might be diagnosed. From an initial weight of 100 kilos it had risen to 117 kilos on 2nd June, but after the injury it fell again to a final weight of 100 kilos.

Cow 3024 in calf also showed transitory diarrhœa on 6th November. On 1st January it calved normally, but suffered some loss in condition and lost weight more or less steadily thereafter. On 8th April another bad attack of diarrhœa set in again, and on 17th April it showed a stiffness and laminitis similar to that observed in the other

cattle. This became more pronounced during the ensuing fortnight, but then passed off, although the animal remained in very low health. In the beginning of May the allowance of coarse fodder (autoclaved straw) was raised to 4 lb. per day, and at the beginning of June, after an attack of diarrhœa and laminitis, to 7 lb. per day. The cow, however, seemed unable to pick up in general health, and was killed for post-mortem on 11th August after ten months of rice feeding. Post-mortem examination merely emphasized the low condition of the animal (slight anaemia), but revealed nothing which could be specifically related to the general failure to recover condition. From an initial weight of 460 kilos this animal had fallen to 395 kilos before calving. A week after calving it showed 334 kilos, and seven months later 270 kilos.

Cow 3039.—The weekly records of this case are not included in the table, since the survival period was so short. The cow was in full calf approaching term, and was started on 14th April on a diet of 8 lb. of rice and 1 lb. of veld hay. On 18th April calving took place, and on 7th May an attack of diarrhœa set in with the concomitant lameness already commented upon. The ration of hay was then raised to 10 lb. to see whether the symptoms would disappear upon a more natural diet. The cow, however, was too far gone to profit, and the symptoms became worse during the ensuing days. On 14th May the beast was unable to stand, and about to die. It was therefore killed for immediate post-mortem. Acute gastro-enteritis was assigned as cause of approaching death. This case, in which the survival period was only twenty-nine days and the diet less open to suspicion of deficiency, is of especial interest in comparison with the others.

Summary of Cattle Tests.—Summarizing these results (Table IX) in the form of approximate totals we have:—

Table IX.

APPROXIMATE TOTALS AND AVERAGES.

ANIMAL.	Young Bull.	Grown Ox.	Young Heifer.	Grown Calf.	Cow in Calf.
Duration of feeding.....	50 weeks	52 weeks	52 weeks	44 weeks	44 weeks
Total polished rice eaten....	5,367 lb.	5,232 lb.	5,326 lb.	1,484 lb.	4,535 lb.
Coarse fodder, veld hay first 29 weeks.....	252 "	239 "	235 "	100 "	257 "
Autoclaved straw, remaining weeks.....	267 "	283 "	291 "	113 "	503 "
Total coarse fodder eaten....	519 "	522 "	526 "	213 "	760 "
Approximate ratio, rice : coarse fodder.....	10 : 1	10 : 1	10 : 1	7 : 1	6 : 1
Approximate average daily intake, rice.....	15.3 lb.	14.4 lb.	14.6 lb.	4.8 lb.	14.7 lb.
Approximate average daily intake, coarse fodder.....	1.48 "	1.43 "	1.45 "	0.69 "	2.46 "
Initial weight of cattle.....	616 "	939 "	737 "	220 "	1,012 "
Alteration in live weight....	-4 %	-10 %	+11 %	—	-41 %

Although these experiments covered periods of from eleven to thirteen lunar months there was nothing in the symptoms of the cattle at any time which could be justly diagnosed as an avitaminosis or anything which could be fairly compared to lamziekte. Beyond periodic diarrhœa, transient laminitis, and a somewhat poor condition of the skin at times, in which a little scurf manifested itself over the neck and dewlap, no characteristic symptoms were detectable in life or revealed on post-mortem from which a specific deficiency disease could be deduced.

The fact that cow 3039 which, as already mentioned, was reduced to the verge of death from gastro-enteritis within a month of commencing the experiment, having shown in aggravated form during life all the symptoms shown by the other cattle surviving for long periods, justifies us in assuming that the transient recurrent laminitis and other symptoms were due to purely digestive disorder. Indeed, we regard it as a matter for congratulation that the remaining cattle were maintained for such long periods upon a concentrated diet containing so small a proportion of coarse fodder. Heifer 2569, which not only survived a full year without loss in weight or condition, but successfully carried a calf through the whole period of pregnancy, is particularly interesting.

It will be noticed from the table that the quantities of rice ingested are high, and that the calorific values of the diets are therefore well above the maintenance requirements of the animals concerned. The quantities of coarse fodder are fairly accurately represented in the table, because the amount supplied was always eaten even when the cattle had to pick out the chaffed material from the admixed rice. The rice figures are not quite so sure, but even supposing that they are uniformly 10 per cent. high, the starch value of the diet corresponds rather to a fattening ration than to mere maintenance requirements, although the protein ration is only maintenance. With, however, so small a proportion of coarse fodder to encourage proper rumination and normal intestinal movement, there is no doubt that during the periods of diarrhœa complete utilization of food did not take place, although during periods of normal defaecation the rice seemed well utilized. To test this point in rough detail, two of the animals, ox 1928 and heifer 2569, which were feeding reasonably well at the conclusion of the experiment and had maintained their original weight (\pm 10 per cent.), were tied up in the stalls for collection of faeces. A probationary period was not necessary, since no alteration was made in the diet, but a few days were allowed to accustom the animals to constant confinement and to get a native boy into the habit of collecting the faeces as soon as voided, without contamination with the urine. Owing to the rough and ready plan of the experiment the intake of rice is not strictly accurate. The faeces, more particularly of the ox, were voided in very irregular fashion, and only the summation results over thirteen days are therefore given. The faeces were so small in amount that the figures for "dry matter" and "total nitrogen" need only be stated, the "N-free extraction," "crude fibre," and "ether extract" ceasing to be of any practical consequence.

The broad figures are embodied in Table X.

Table X.

	Heifer.	Ox.
Approximate dry matter of rice eaten.....	168 lb.	98 lb.
Containing total nitrogen.....	2.06 "	1.20 "
Approximate dry matter of autoclaved straw.....	22 "	22 "
Containing total nitrogen.....	0.14 "	0.14 "
Approximate dry matter of ration.....	190 "	120 "
Approximate total nitrogen of ration.....	2.20 "	1.34 "
Total dry matter of faeces.....	28 "	14 "
Total nitrogen of faeces.....	0.59 "	0.39 "
Hence digested dry matter of ration.....	162 "	106 "
Hence digested total nitrogen of ration.....	1.61 "	0.95 "

It is seen that the faecal dry-matter is very low and the faecal nitrogen relatively high. Calculating the undigested nitrogen to be expected in the faeces from Kellner's tables we have 0.37 lb. for the heifer and 0.25 lb. for the ox, as against 0.59 and 0.39 actually found. The reason for this is doubtless the high proportion of digestible starch and low proportion of coarse fodder in the ration, resulting in faeces small in amount and in which a relatively large proportion of the nitrogen is derived from bacterial, and intestinal epithelial, debris. The heifer ate readily enough during the thirteen days' period, but the ox considerably less than his average over the preceding experimental year. The main point of interest, however, is the fact that the digestion of the ration in which granular concentrated food (rice) preponderated so heavily over coarse fodder throughout a whole year is, in the absence of diarrhoea, quite normal. There can therefore be little doubt that not only the intake but also the extent of its digestion amply sufficed to cover ordinary metabolic requirements. This is, of course, in most cases obvious from the live-weight records of the cattle themselves.

The fact that young bull 3108, although ingesting a quantity of food sufficient in the ordinary way for continued growth, did not increase in weight has already been commented upon. The case of calf 2758 is also of interest. Considering only the period from 7th October, 1913, to 2nd June, 1914, before the accident, this animal increased 17 per cent. in weight, but this rate of increase is abnormally small. The protein ratio of the rice-hay ration is, of course, low (about 1:13), but how far the failure of these two cattle to grow normally is accounted for by the low protein supply *per se*, how far attributable to a specific deficiency either in amino-acid "Bausteine" (improbable), or "growth-vitamine," and how far explainable on grounds of mechanical unsuitability of the ration, is a matter for speculation. The question also of the mineral requirements for growing cattle did not come within the scope of the present investigation, although a rice ration is certainly defective in this respect. Later on we hope to carry out investigations more specifically dealing with the problem of growth in stock.

The main point, however, which the experiments were designed to illuminate we regard as sufficiently clear. The majority of the animals survived the digestive disturbances incident to the mechanical nature of the diet, and in spite of its *extremely low vitamine content*,

developed nothing definable as an avitaminosis within a period of so long as one year, and showed no tendency to contract lamziekte. The very low vitamine content of the ration will be readily admitted *a priori*.

The pigeon experiments showed that a rice-hay or rice-straw mixture of ration, rice : fodder, 2:1, was inadequate to ward off polyneuritis gallinarum. The rice-fodder mixture in ration 10:1 represents only one-fifth of the pigeon proportion of fodder. The effect of autoclaving the coarse fodder at 125°-130° for three hours could not be estimated owing to the fact that the pigeon method failed to clearly detect vitamine in workable quantities of untreated material, but even if it be supposed that the autoclaving in no way affected the hypothetical vitamine content of the straw, the amount supplied was so small that it could not materially supplement the polished rice. At the same time the supposition involves a very presentable degree of stability of the vitamins in grass and diminishes the likelihood of their destruction under natural conditions.

The reason why no avitaminosis was manifested in our experimental cattle is a matter for speculation.

It may be that an avitaminosis could be produced in, say, two years of deficient feeding instead of one, but we doubt it. It seems fairly certain that the exogenous vitamine requirements of cattle are extremely small. It may be that the residual vitamine still present in the highly-polished rice (P_2O_5 content 0.21 per cent.) was still sufficient for bovine metabolism. It may be that cattle are capable of synthesising their own vitamins in virtue of the extensive bacterial flora of their intestinal tract—a sort of commensal symbiosis. If vitamine of some sort is required in the metabolism of cattle in very small amounts, and there is no valid reason for assuming that it is not, we think it not unreasonable to suspect the intestinal organisms of covering a possible exogenous deficiency in the rice. The capacity of lower organisms to synthesise vitamine is well known (yeast particularly). When we consider that the bacterial contribution to the faeces in various animals on highly digestible diets has been assessed by various authors at from 10 per cent. to 50 per cent. of the total dry matter (of human infants about 70 per cent., Hecht¹², and of rats 23-42 per cent., Osborne and Mendel¹³), consider the lengthy time during which digesting food masses remain in the intestinal tract of cattle, and consider that the bacterial débris actually found may be largely from late generation unresolved organisms, there seems room for the assumption that a very extensive cycle of bacterial growth and decay can complete itself within the intestines and conceivably supply the fraction of a gramme of vitamine probably necessary in bovine daily metabolism.

Whatever be the explanation of our experimental facts, their practical significance seems fairly clear. We do not think that any natural veld which still retained a nutritive value high enough to cover the ordinary crude metabolic requirements of grazing cattle would ever be at all likely to fall below our experimental diet of polished rice and autoclaved straw in respect to the subtler demands for "minimum substances." We do not think that the grazing of the lamziekte area, where the cattle are seldom forced to depend solely

¹² Hecht. "Die Faeces des Sauglings und des Kindes," 1910.

¹³ Osborne and Mendel. "The contribution of Bacteria to the faeces after feeding diets free from indigestible components." Journ. of Biol. Chem., Vol. XVIII, No. 2, 1914.

upon the vegetation surviving for more than one absolutely rainless year, is likely to be more deficient in vitamine than in more general nutritive respects. We rather think that cattle on poor veld would die of poverty before succumbing to a specific avitaminosis.

These points will be brought up again in Section VI and correlated with the evidence of the field experiments.

3. RICE-FEEDING OF HORSES.

Owing to the lesser importance of horses for our primary experimental purpose, only two animals were used. As in the case of cattle, soaked rice with a small variable proportion of veld hay or autoclaved straw constituted the ration. The first horse, No. 7403, weighed 442 kilos at the beginning of the experiment, dropped to 420 kilos during the first week, and thereafter fell steadily to a final weight of 380 kilos at the end of 16 weeks, then dying with the symptoms of colic and showing post-mortem lesions of acute gastro-enteritis and slight hydropericard, but having shown no symptoms of a deficiency disease during life. During the four months of survival, 1800 lb. of polished rice and 175 lb. of veld-hay were eaten, i.e. approximately ten times as much rice as hay by weight. There is no doubt that a fair proportion of the rice eaten was voided undigested, and little, if any, significance can be attached to the fall in weight of 10 per cent. between the first week and the last.

The second experiment is more interesting owing to the longer dietetic period and the constancy in weight. This young horse (about 2 years), No. 6575, put into experiment on 10th February to replace No. 7403, was carried on until 12th August, when the experiment had unfortunately to be abandoned. The initial weight of the animal was 222 kilos and the final weight 220 kilos, minor fluctuations occurring from week to week. Throughout the whole period of 182 days, the gross quantity of rice eaten was 1480 lb. The coarse fodder eaten amounted to 58 lb. of poor quality veld-hay during the first 10 weeks and 224 lb. of autoclaved oat-straw during the last 16 weeks, or about 200 lb., allowing for 10 per cent. of moisture generally taken up in autoclaving. Omitting the preparatory period of the experiment, during which a little oats was mixed with the rice to encourage feeding, we have a record for this horse of 6 months on a diet in which polished rice made up about 80 per cent. by weight, or about 95 per cent. of the total calorie value of the ration, during which time the horse maintained its general health and body-weight at the original level and showed no symptoms whatsoever of any deficiency disease, although a distinct laminitis appeared on two occasions throughout the feeding period. We have therefore to assume either that an avitaminosis in the horse is a matter of very slow development or that the vitamine requirements, in respect to a full gross metabolism are covered by the residual traces of vitamine in the highly polished rice or present in the small quantity of coarse fodder supplied.

It had been intended to carry the feeding of this horse further, but, although circumstances forbade this, we think the experiment of definite value in at least showing that an avitaminosis in the horse is not likely to crop up under practical conditions of feeding however stingy these may be. On a poor veld the horse, owing to its lower capacity for digesting coarse fodders, is less likely than the ox to weather through a long period on low-grade dried grass. The horse,

too, in its labouring existence, is more likely to meet some variety in its food, and it is difficult to picture a ration upon which a horse would receive less vitamine than that contained in our experimental diet.

4. RICE-FEEDING OF SHEEP.

Soaked rice was fed as before, but, owing to the method of pen-feeding adopted, the individual intake is not accurately known. The object of the experiment merely involved the attempt to produce an avitaminosis, and the same labour in determining the amount of rice eaten was not expended in the case of the sheep as in that of the cattle. The rice supplied was weighed out (dry), but the residue uneaten could not be accurately estimated owing to scattering and tramping in the pen. At first the attempt was made to feed on rice alone, but, after the occurrence of three deaths from enteritis, a small ration of fodder was given. From 23rd December, 3 oz. of veld-hay was allowed, this being replaced by $\frac{1}{2}$ lb. of sterilized oat-straw at the end of April.

A general commentary on the individual cases may be given for convenience in gauging the significance of the results.

Sheep 4143 died after a fortnight's feeding, showing a fall in weight of 23 per cent., and a post-mortem crediting enteritis as cause of death.

Sheep 4144 died after a month on the rice diet, also showing a decrease in weight of 25 per cent. and post-mortem evidences of enteritis.

Sheep 4148 survived 54 days with a 27 per cent. decrease in weight. Post-mortem, enteritis and anaemia.

Sheep 4145 was still well on 22nd December at the time of introducing the daily allowance of 3 oz. of hay, although at this time it had fallen 15 per cent. in weight.

This small amount of coarse fodder apparently made a considerable difference to the diet (mechanically?), since the new sheep, 5152, 5149, 6256, survived for very much longer periods than the earlier ones. Sheep 4145 itself survived until 19th July, then dying in a very emaciated condition and showing a decrease in weight of 38 per cent. Post-mortem, enteritis. An administration of 42 grammes of orypan, one-half drenched and the other half given subcutaneously the day before death, had no effect whatever. Survival, 270 days.

Sheep 4146 survived 254 days, also showing an emaciated condition at death and a drop in initial weight of approximately 40 per cent. Post-mortem, inanition and acute enteritis.

Sheep 4157 survived 77 days from the beginning of the experiment, decreasing 17 per cent. in weight. Orypan, 20 c.c. in saline, was injected intravenously, but without effecting improvement, although the quantity given was about the curative dose for this sheep reckoned on the basis of live-weight from the pigeon tests. Post-mortem, enteritis and pericarditis.

The remaining four sheep, 5152, 6088, 5149, 6256, were put out of experiment on 8th August, the duration of feeding being 37 weeks, 34 weeks, 37 weeks, 30 weeks, and the decrease in initial weight 49 per cent., 33 per cent., 47 per cent., and nil, respectively.

In no case could any symptoms be detected in any way comparable to beriberi or scurvy in the human or to polyneuritis in pigeons.

We have to admit that there are factors, such as mechanical disturbance due to the physical nature of the food fed, which require

elucidation. That this physical factor was a disturbing element is suggested by the enteritis invariably occasioned, and perhaps also by the fact that one sheep fed for a period of over a week with pressed yeast at the rate of over 2 grammes per kilo, and subsequently treated with a theoretically curative dose of orypan, showed no improvement in condition. The further fact that the sheep tolerated the rice diet much better after addition of 3 oz. of veld-hay on 22nd December, and still better after the substitution of 8 oz. of autoclaved straw for this after 25th April, points in the same direction, although the possible beneficial effect of vitamine in the coarse fodder is not excluded. Doubtless it would have been better to have kept the fodder still lower and to have rendered the rice more suitable mechanically by boiling.

The four animals surviving for periods of 30 to 37 weeks, of which two received rice alone for the first five weeks, are interesting in at least showing that an avitaminosis is extremely difficult to produce in sheep. Sheep 6256, which received the small fodder ration from the beginning and apparently escaped the enteritis affecting the others, shows no drop in weight at the end of seven and a half months. This particularly interesting case brings out the fact, even on the assumption that polished rice alone would have been a "deficient diet," that the exogenous vitamine requirements of the sheep are extremely low, a daily allowance of approximately 5 or 6 oz. of coarse fodder (average) being apparently sufficient to meet the vitamine deficiency in the rice. Considering the pen records of rice eaten and the fact that this particular sheep ate well, we may assume an average daily intake of $1\frac{1}{2}$ lb. of dry rice. We then have an instance in which a sheep retained its original condition and weight over a period of $7\frac{1}{2}$ months on a rice ration in which the possible deficiency is apparently balanced by one-fifth of its weight of coarse fodder. The sheep experiments are, however, on the whole, distinctly ambiguous. It is difficult to know how much stress to lay upon the cases of death, since they were all complicated by an enteritis *not necessarily* associated with deficiency. The fact that the symptoms of the sheep could all be explained without recourse to the supposition of an avitaminosis, and also the lengthy survival of sheep 6256, make interpretation difficult. The pen records do not allow of an accurate analysis of the calorific intake of each animal, and it is certainly true that some of the sheep showed marked disinclination to eat during the later stages of feeding. It is therefore difficult to say how far the marked decrease in weight observed in most cases is due to inadequate intake, how far to general lowering of condition as the result of mechanical enteritis, and how far to a specific deficiency in the diet with concomitant, possibly symptomatic, enteritis. The possible influence of a change in the general balance of intestinal flora is also a factor not to be ignored.

5. RICE-FEEDING OF GOATS.

The experiments with goats (Angora) are similar to those with sheep. As before, the object was merely an attempt to produce an avitaminosis in the goat, and the trials were simply arranged within the limits of available accommodation, labour, and time. After a brief preparatory period during which a little mealie-meal and bran were given to accustom the animals to the rice diet, soaked polished rice was exclusively fed for two months. The occurrence, however,

of three deaths from enteritis decided the addition of 3 oz. of veld-hay to the daily rations, and subsequently the substitution of this by $\frac{1}{2}$ lb. of autoclaved straw at the end of April. This, doubtless, lowered the value of the tests from the point of view of pure deficiency dietetics, but practical information as to the effect of a vitamine-low ration was still hoped for. Unfortunately, the factors weakening the results on sheep also entered here, but revision of the experiments has been postponed to a more convenient time.

The weekly protocols of live-weight and rice eaten need not be detailed, since the intake for pens, and not for individual goats, was recorded.

The following summary, Table XI, may be taken for purpose of discussion, any desirable detail being mentioned in the test.

Table XI.

NUMBER OF GOAT. }	3231	3397	5001	5037	5307	5318	5327	5328	5335	5336	5338	5343	5569	5575	5578
Survival in days	186	186	112	98	35	60	50	61	50	266	112	250	58	71	40
Percentage fall in initial weight	23	13	47	29	31	23	31	40	12	42	41	19	29	38	21
Post-mortem evidences	Still alive.	Still alive.	Still alive.	Cholecystitis.	Anaemia and inanition.	Enteritis and anaemia.	Anaemia and inanition.	Anaemia and inanition.	Enteritis, icterus, and anaemia.	Acute enteritis and inanition.	Anaemia and inanition.	Still alive.	Catarrhal enteritis and anaemia.	Anaemia, icterus, fatty degeneration of liver.	Gastro-intestinal catarrh and icterus.

From post-mortem examination the cause of death was in most cases put down as inanition, and although enteritis was usually also observed, it was seldom so marked as in the case of the sheep. No other constant lesions were found, and during life no symptoms were shown which necessitated the assumption of specific rather than general malnutrition. The method of pen-feeding precludes actual assessing of the true calorific intake, and the rice actually ingested by a goat observed to be eating little rice, and then either resuming normal intake or dying from the pen in which the remaining goats were eating freely, cannot be traced. There is therefore no way of properly determining how far the malnutrition was due to a deficient intake and digestive disturbance and how far to a specific deficiency in the diet, i.e. how far the malnutrition resembles that of pigeons ultimately dying of weakness on a rice diet of enforced ample intake. The most interesting animals are naturally those which survived for long periods, and which therefore did ingest an amount of food at least sufficient for bare metabolic requirements.

These animals are numbers 5343, 3231, 3397, and 5336, surviving approximately 8 months, 6 months, 6 months, and 9 months respectively. Number 3397 is most interesting from our point of view, since it practically maintained its weight throughout the experiment. A drop in weight from 115 lb. to 105 lb. occurred about the third week

of the experiment, but thereafter, for the ensuing five and a half months, the fluctuations around the latter figure were inconsiderable, the goat being finally put out of experiment at a weight of 100 lb. Goat 5343 only dropped from 80 lb. to 65 lb. in 8 months of rice-feeding, and was far from death by starvation when put out of experiment on 9th August. Goat 3231 also showed its decrease in weight within the first two months, remaining thereafter more or less in equilibrium.

Neither of these two justify the supposition that the ration was specifically deficient. No. 5336, however, showed a decrease of 42 per cent. on its initial weight, and if the enteritis be regarded as symptomatic of the chronic form of a deficiency disease it might justly be compared to the case of pigeons dying of chronic polyneuritis in atrophic form. On the other hand, the early deaths of goats, such as No. 5307, rather suggests that the enteritis is mechanical, and that the fall in weight is independent of specific vitamine-deficiency.

We are at least entitled to conclude that an avitaminosis in the goat is difficult to produce, and that the vitamine requirements of this animal are probably very low.

6. RICE-FEEDING OF PIGS.

Three pigs were fed on *rice alone* after a short preparatory period during which a small quantity of maize-meal was given to encourage the animals to eat freely. The rice was *boiled* for this experiment.

Pig 205, weighing 47 lb., died at a weight of 40 lb., 51 days after commencement of feeding, but since swine-fever was diagnosed as cause of death, the test must be discarded as invalid.

Young pig 210 died after 136 days of rice-feeding, showing a drop in weight from 65 lb. down to 50 lb., the decrease taking place in the last three weeks. Post-mortem revealed cholelithiasis as well as gastro-enteritis, so that it is doubtful whether deficiency of the rice diet can be held responsible for the death.

Pig 209 survived 160 days. For this animal an individual pen-record is fortunately available for the later and most important months of feeding, and there is no doubt that a calorie intake well above maintenance requirements was regularly ingested right up to within a few days of death. The weekly live-weight records showed merely slight fluctuations around the original figure of 150 lb. until the last week, when a pre-mortal drop of 15 lb. was registered. Post-mortem revealed no lesions other than enteritis to account for death.

Ignoring pig 205 we are left with two animals surviving 19 weeks and 23 weeks respectively on an exclusive diet of polished rice. Assuming the cholelithiasis observed in pig 210 as partly responsible for death irrespective of the system of nutrition, we are left with pig 209 with its survival of approximately six lunar months to account for. No symptoms of any *specific* deficiency disease were shown and, as already indicated, the body-weight was maintained right up to the week of death, and then only dropped 10 per cent. It is doubtful whether this case can be accepted as an evidence of vitamine-hunger, but since no other cause of death can be assigned beyond enteritis, which itself may be symptomatic of deficiency disease in the pig, the question must be left to more detailed future work to decide.

There is, however, another point of some interest in connection with the weight records of all three pigs. On a fully adequate diet all three should have shown substantial increase in weight during

the experimental periods of 51, 132, and 160 days respectively, instead of which they either remained at original weight or showed decrease, although eating well above maintenance requirements. We are disposed therefore to conclude that, irrespective of the development of a theoretically possible avitaminosis, polished rice is not an adequate diet for the growth of pigs. In this connection the experiments of McCollum and of Hart and McCollum¹⁴ on rations restricted to the maize or wheat grain are of interest. We hope to investigate this point further, but meantime have no evidence upon which to base discussion of the validity of this supposition or to decide whether the apparent growth failure observed is due to deficiency in vitamine or in inadequate content of essential amino-acid units of rice protein (improbable), or in mineral constituents, or in all three. We propose to shortly carry out a further series of dietetic experiments, and by working on fewer animals to be able to devote more attention to the different factors involved in health maintenance and growth, and to add histological study to the scheme of work.

7. FEEDING TESTS ON DOGS.

These experiments were carried out with a triple purpose (*a*) to control the dietetic experiments with lamziekte biltong on pigeons, with animals for which meat is a natural food, (*b*) to detect, if possible, any effect which might arise through feeding the diseased flesh, (*c*) to produce an avitaminosis (if possible) in the control dogs fed on rice alone. At the same time, owing to the wide rather than detailed plan of work, the precise individual record of intake of each dog was not determined.

The animals were put up in batches of three or four, and although the gross quantities of rice eaten are approximately known, individual data are only available in the case of single dogs surviving their kennel-fellows. Unfortunately also, although all dogs were weighed every week, the records are not so instructive as they might be owing to the fact that many of the animals (obtained through the police authorities) were in a half-starved initial condition, and much below the weight they would have shown in well-fed condition.

(*a*) *Rice-feeding*.—The following summary of data from the rice controls may suffice—Table XII.

Table XII.
DOGS FED ON BOILED RICE ALONE *ad lib*.

No. of dog.....	1256	1249	1269	1270	1252	1254	1257	1258	1259	1260	1261	1262	1267
Survival, in days.....	69	95	42	102	50	63	46	92	69	58	47	55	116
Initial weight, in lb....	25	41	20	25	53	45	45	33	25	31	35	30	30
Final weight, in lb....	20	28	15	15	40	30	33	17	15	15	24	25	18
Percentage decrease, in weight.....	20	32	25	40	25	33	27	49	40	51	31	17	40

¹⁴ Hart and McCollum. "Influence on growth of rations restricted to the corn or wheat grain." Journ. of Biol. Chem., Vol. XXX, No. 3, November, 1914.

On the whole the rice was eaten fairly well for the first month or two, and from the kennel records it would appear that a full calorie intake was obtained. Thereafter the dogs fed irregularly, eating one day and not the next, two dogs in a cage eating fairly well while the third perhaps ate little or nothing. An aversion to rice was certainly shown, but this in itself is more or less natural, considering the wet vegetarian nature of the diet. As signs of weakness and malnutrition advanced, however, the aversion became more marked, and the fact that a dog on the verge of starvation would occasionally refuse the boiled rice altogether indicates a genuine "Reisekel," and not merely a stupid prejudice against vegetarian principles. For a short time before death many of the animals ate practically no rice, and it is probably true to say that the dogs succumbing first were those in the initially poorest condition.

Post-mortem examination of the dogs at death showed only varying degrees of inanition with, in some cases, minor inconstant lesions of no special significance. In no case could the lesions either of beriberi or scurvy be macroscopically established, and during life no symptoms suggestive of a neuritis could be definitely diagnosed. One or two cases of minor skin lesions were at first suspected as incipient scorbutic manifestation, but were, after careful examination, finally dismissed as irrelevant.

One case which two days before death showed the nearest approach to symptoms which might possibly fit in with an avitaminosis may be described in detail. This is represented by dog 1256. It was in very reduced condition, and persistently lay on its side in its kennel. When lifted, the fore legs knuckled over, and the dog adopted a sitting posture from which it turned a complete somersault backwards. The hind extremities were almost insensitive to pricking, and the dog seemed to have only imperfect control of its muscles. Thereafter it remained on one spot, lying in its excreta without moving, occasionally sitting on its hindquarters with the fore legs extended and muscles quivering. Slight abrasions of the skin over the rumps were largely mechanical. In this condition it survived two days, consciousness being clear throughout.

Post-mortem.—Hairless patches on quarters and scrotal regions, but of doubtful significance. Dilation of the right heart, slight pulmonary congestion and hyperaemia, small hyperaemic areas in stomach, colon, and duodenum, ecchymosis of small intestines; slight hyperaemia of pharynx and injection of the vessels of the trachea. Otherwise normal.

Dogs 1249, 1270, 1258, 1267, showing survival periods of from three to four months, are naturally the most interesting. These animals, weighing 41, 25, 33, 30 lb. respectively, had, after six or seven weeks, dropped to 30, 15, 20, 20 lb. In the ensuing weeks these weights were maintained with minor fluctuations up till death.

In the earlier periods these dogs ate up to 1 lb. of rice each, which certainly provided a full calorie intake, and throughout the experiment ate comparatively well. Dogs 1249 and 1258, for instance, ate about $\frac{1}{2}$ -lb. of rice a day during the last fortnight of life, and cannot therefore be said to have died of starvation. At the same time, as already remarked, no symptoms of a *specific* deficiency disease could be observed, and the nature of the dietetic records was not sufficiently detailed to absolutely exclude the supposition that death,

in the case of the majority of the animals experimented upon, was not partially due to a chronic under-nutrition in the more ordinary sense. That, however, rice is not a complete food for dogs we have no doubt, and are inclined to regard both the disgust towards rice displayed by most of the animals, and their ultimate death, as specifically related to the absence of vitamines in the diet. It can at least be said that even *if* rice is dietetically adequate, the dogs still refuse to eat enough to stave off starvation.

(b) *Rice plus dried flesh*.—Pigeon experiments having already shown the low antineuritic value of dried flesh, it was considered of interest to compare normal biltong, lamziekte biltong, and stijfziekte biltong, when fed as supplementary rations to dogs on a polished rice diet.

Normal biltong.—Two dogs, Nos. 1275 and 1276, were given 4 ounces of ordinary butcher's biltong in addition to a daily supply of boiled rice. In 56 days dog 1275 rose from an initial weight of 43 lb. to a final weight of 55 lb., while dog 1276 remained at fairly constant weight of 58 lb. throughout. Both were in such excellent condition and appeared so healthy that it was not considered necessary to carry this experiment (control) any further. In addition to the biltong the dogs ate rice freely, in amount about as much as the dogs on rice alone at their best.

Lamziekte biltong.—Four dogs in fair condition were put on to biltong made from cattle which had died of lamziekte at Armoedsvlakte, 1 lb. per dog per day being allowed—of which nearly all was invariably eaten.

No. of dog.....	1265	1266	1268	172
Initial weight, in lb.....	27	30	25	31
Weight after 44 days, in lb.....	30	32	30	33

The condition and weight of the dogs improved. Fed alone, therefore, lamziekte biltong amply sufficed for nutrition, and produced no ill effect in any respect.

After 44 days the biltong was withdrawn and the dogs fed on boiled rice. Dog 1265 rapidly fell in weight, although it ate rice readily at first. It died with the general symptoms of under-nutrition after 49 days, having decreased in weight from 30 to 20 lb. Dog 1272 survived 70 days, dropping to 20 lb. Dogs 1266 and 1268 were taken out of experiment after 88 days in very low condition and weighing 20 lb. and 19 lb. respectively. The rice eaten by these two dogs during the last few weeks averaged about $\frac{1}{2}$ lb. a day each, so that the "under-nutrition" cannot be wholly explained as a simple starvation process, although no specific avitaminosis was discernable.

Lamziekte biltong $\frac{1}{4}$ lb., rice ad lib.

No. of dog.....	1277	1278	1279	1280
Initial weight, in lb.....	30	25	17	25
Weight after 130 days, in lb....	41	28	25	30

It is seen that the dogs gained considerably in weight. They also improved materially in general appearance. The rice supplied was eaten freely throughout the experiment.

A fifth dog (1264) in a rice-feeding test for 56 days, during which time it had fallen in weight from 34 lb. to 26 lb., was allowed $\frac{1}{4}$ lb. of lamziekte biltong in addition to rice. After 88 days it was put out of experiment in excellent condition at a weight of 40 lb.

From these experiments we may safely conclude that lamziekte biltong is not only itself dietetically efficient, but when present in a rice ration to the extent of less than one-third, is capable of supplying any deficiency which may exist in the rice itself.

At the same time it may be noted that no disease is producible in dogs through prolonged feeding on the biltong made from diseased animals.

(c) *Stijfziekte Biltong*, $\frac{1}{4}$ lb., plus rice *ad lib*.

No. of dog.....	1281	1282	1283	1284
Initial weight, in lb.....	35	25	20	25
Weight after 56 days, in lb.....	36	20	23	25

The dogs maintained their initially good condition throughout the experiment, and, with the exception of No. 1282, showed little change in weight. Rice was eaten freely without aversion, and it was not considered necessary to continue this trial. The conclusion drawn in respect to lamziekte biltong applies, *pari passu*, to stijfziekte biltong.

IV. FIELD EXPERIMENTS ON THE LAMZIEKTE AREA.

As indicated in Section II, the object of these experiments was, in the main, the converse of the laboratory rice-feeding tests, i.e. in the field tests cattle were allowed to graze over lamziekte veld and various foodstuffs rich in vitamine were supplied as supplementary rations, to determine if the natural occurrence of the disease could be thereby prevented. At the same time control animals were grazed without supplementary rations, and to one batch a supplement of rice, in amount sufficient for major metabolic needs, was given with the idea of still further reducing the vitamine supply of the cattle concerned. An observation previously made, that cattle in good condition were not only as readily afflicted with lamziekte but actually appeared more susceptible than ill-nourished animals, suggested the inclusion of supplementary rations designed not so much with the intention of supplying vitamine as with the idea of merely improving the general condition of the cattle. A feeding test was also carried out in which hay cut from control Camp C was fed to stalled cattle, thus eliminating the factor of free movement over the veld.

For the mere purpose of facilitating the task of obtaining a full calorie intake, treacle and linseed-oil were used. For the specific purpose of supplying vitamine, and with the incidental object of improving the general condition of the cattle, bran, beans, maize, potatoes, and a mixture of the first two with a modicum of molasses, were used. With the object of testing the prophylactic and therapeutic value of yeast, quantities of this material were supplied by drenching.

The potato supplement was included so as to remove the possible reproach that only the vitamin of storage grains was supplied and not the probably more labile vitamin of watery foods. Turnips were not available, and lucerne involved an inconvenience in transport and in the matter of regular supply in the green state.

Since the object was to secure as many cases as possible within limited time, the cattle used were drawn chiefly from areas in which lamziekte had occurred at one time or another, and placed in camps at Armoedsvlakte, a farm near Vryburg, specially selected for its reputation as a very bad lamziekte area. The animals were divided into lots of ten, kraaled at night, fed with the supplementary rations in the early morning, and turned out to graze for the rest of the day.

Owing to the varying dates upon which the experiments were commenced and the slight changes in plan from time to time, it will be clearer if brief seriation comments are made before summarizing the data.

Treacle, Lot C, 10 cattle.—Daily ration of $2\frac{1}{2}$ lb. per head, smeared upon hay and fed in troughs. Commenced 18th December, 1913, discontinued 10th August, 1914.

On 8th April, about four months after commencement, cattle 2976 and 2996 were noticed sick. No. 2976 died on 9th April, and 2996 on 13th April, both of lamziekte. On 16th May animal 2989 sickened. It was treated with 100 grammes of orypan, but showed no improvement, dying 4 days later of acute lamziekte.

On 20th May animal 2623, which had replaced animal 2996, developed an attack. Ten days later, when on the verge of death, it was put out of misery, and the diagnosis confirmed by the negative blood-smears and post-mortem findings.

On 30th May the quantity of treacle fed was doubled, but no further cases happened to occur up to the date of discontinuance.

Linseed-oil, Lot D, 10 cattle.—The daily ration of $\frac{1}{2}$ lb. per head was also fed smeared on hay. Commenced 6th January, 1914, discontinued 10th August, 1914.

On 7th February animal 2343 sickened, and died 2 days later. On 8th March No. 2614 developed acute lamziekte, dying the following day. On 30th May the quantity of oil was doubled, but no further cases occurred until 11th June, when a mild evanescent attack was diagnosed in animal 2353.

Beans, Lot E, 10 cattle.—Daily ration of $2\frac{1}{2}$ lb., fed as soaked whole beans at first, later as coarsely ground meal. Commenced 18th December, 1913, discontinued 10th August, 1914.

On 11th February, animal 2840 was diagnosed as suffering from a slight attack of lamziekte, but it recovered a little later. On 30th May, however, it developed an acute attack and died in 2 days. No. 2843 also sickened on 5th May but recovered. On 30th May, the daily ration was raised to the very liberal allowance of 4 lb. per head per day, but nevertheless this animal developed a second attack on 28th June, of which it died on the 30th.

Incipient affliction was diagnosed in No. 2993 on 18th May. Three days later acute lamziekte set in with rapid death. Animal 3170 died of lamziekte on 13th June, 14 days after the bean-meal supplement had been raised from $2\frac{1}{2}$ lb. to 4 lb.

Bran, Lot G, 10 cattle.—Daily ration of $2\frac{1}{2}$ lb. per head, raised to 8 lb. on 28th May, generally given dry. Commenced 18th December, 1913, and discontinued 10th August, 1914.

The first case occurred on 5th January, on which day heifer 2139 went down, death ensuing the day after. On 13th March animal 2145 showed signs of lamziekte and was treated with orypan. It recovered, but the recovery is believed to have been spontaneous. This point will be mentioned again in the subsequent discussion.

In May three more cases occurred. No. 3166, which had passed through a slight attack in February, succumbed to an acute attack on the 29th and died within twenty-four hours. On the 19th, animal 2998 developed acute lamziekte, dying on the day following. On the 30th No. 2999 was suspected, but it recovered within a few days, leaving the diagnosis doubtful.

Mixed Ration, Lot F, 10 cattle.—Commenced 6th January, 1914, discontinued 10th August. During the first month the total supplementary ration for all ten animals consisted of 25 lb. bran, 9 lb. of bean-meal, 6 lb. of treacle; or 4 lb. of the mixture per head per day. Thereafter the bran was reduced to 10 lb. until the end of May, at which time the whole ration was raised to 20 lb. bran, 20 lb. of bean-meal, and 5 lb. of treacle.

Animal 2974 died of lamziekte on 7th February. On 13th May two cases occurred, both terminating fatally within 2 days—No. 2352 dying on the 14th and 2987 on the 15th. In the former case treatment with orypan (see later) was carried out, but with no effect. About 20th May, animal 3145 sickened, but recovered spontaneously during the ensuing week.

Crushed Mealies, 10 cattle.—13th June to 10th August. Daily ration of 3 lb. per head allowed. This test, it will be noticed, was commenced late. Within the experimental period of two months only one case occurred—animal 3192 dying of acute lamziekte on 26th July after having eaten 130 lb. of maize in the 43 days of survival.

Potatoes, 10 cattle.—19th March to 10th August, 1914. The daily ration of 14 lb. per head was roughly crushed immediately before feeding. On 1st May animal 2962 was diagnosed as a doubtful case, but the attack passed off. On 12th May No. 2383 succumbed to per-acute lamziekte. On 31st May animal 2512 developed a slight attack but recovered. On 5th August No. 2962 contracted an acute attack and died the following day.

Yeast, 10 cattle.—The pressed brewers' yeast was diluted with water, boiled for a few minutes, allowed to cool, and drenched. The experiment was started on 29th March, but owing to a slip in the schedule the daily quantity of yeast given at first was $\frac{1}{4}$ oz. instead of $\frac{1}{2}$ lb. This, however, was rectified after the lapse of three weeks, and 5 oz. per head were given each alternate day from 21st April to 25th May, after which 5 oz. were given every day. The validity of tests with quantities of this size will be discussed presently. Animals 2488 and 2603 developed acute lamziekte on 1st May, dying within 2 days.

On 18th May No. 2349 was diagnosed as suffering from lamziekte, and on 21st May it died. On 23rd May animal 2615 died after 2 days' illness. No. 2338, put in on 26th May to replace 2615, succumbed to per-acute lamziekte on 14th June.

Rice.—Five cattle were started on 5 lb. of rice per head per day on 6th January. On 16th May five more cattle were added, and on 30th May the daily ration for all animals was increased to 10 lb. of rice per head. The rice was given soaked.

Animal 3137 contracted lamziekte on 20th March and died on the 26th. On 19th May No. 3141, of the second batch, after only 3 days on rice, was noticed somewhat stiff in gait, but this rapidly passed off again and was not diagnosed as lamziekte.

Animal 2367 died of lamziekte on 24th June.

Cow 3134, calved on 26th May, developed a severe attack on 28th June, and was killed for post-mortem (and fresh histological material) on 3rd July. Animal 2965 also died of lamziekte on 3rd July after a few days' illness.

A fifth case (2441), which died 2 days after being put into experiment, and was replaced by a fresh animal, is excluded from consideration, as in the initial stages of disease at the time of commencing.

The remaining six cattle were not affected up to 10th August, the date of discontinuance. The digestive troubles so prevalent amongst the laboratory cattle were evidently prevented by the *ad lib.* grazing in the field.

Controls, Lot A.—22nd January to 10th August, 1914. Fourteen cattle grazing night and day in the same camp, made up the controls.

Four cases occurred. No. 3148 went down on 13th May, dying on the 14th in spite of orypan treatment. Animal 2328 was diagnosed as suffering from lamziekte on 21st May and died on the 24th. No. 3151 developed an attack on 3rd June, was treated with orypan on the 4th, but died shortly after. Animal 2323 succumbed to acute lamziekte on 13th June.

A fifteenth animal brought up from Kaffraria on 5th May developed lamziekte on 11th June. This case is interesting owing to the shortness of the period on the area before the attack ensued, but its significance is diminished by the fact that the district from which the animal was drawn is also a reputed lamziekte area.

For purposes of general survey, the following tabular summary (Table XIII) of results may be given, only those cases ending fatally being considered. Animals replacing fatal cases and themselves subsequently succumbing are included, although the date of their entrance into the experiment is for convenience omitted from the table. The last column, representing roughly the proportion of the total digestible starch equivalent of the gross feed which was supplied by the supplementary rations, is based upon the liberal assumption that the cattle (ranging round 700 lb. live-weight) in grazing naturally, manage to get veld herbage corresponding to about 7 lb. "starch-equivalent" daily. The starch-equivalent of the supplementary rations, taken from Kellner's or Crowther's tables, is then roughly calculated for the gross amount of supplement eaten over the known number of days, and expressed as a fraction of the assumed total starch-equivalent.

Table XIII.

Experiment Com- menced.	Experiment Dis- continued.	Duration of Experiment.	Mortality from Lanzietke.	Number of Animals Concerned.	Date of Death, 1914.	Days of Feeding before Death.	KIND AND QUANTITY OF SUPPLE- MENTARY RATION EATEN.		
							Kind.	Approximate Total Quan- tity Eaten.	Computed Fraction of Maintenance.
Dec. 18, 1914	Aug. 10, 1914	235 days	4 out of 11	2976 2996 2989 2623	April 9 April 13 May 20 May 30	112 116 153 37	Treacle	280 lb.	About 1-7th.
								290 "	
								380 "	
								90 "	
Jan. 6, 1914	Aug. 10, 1914	216 days	2 out of 10	2343 2614	Feb. 9 March 9	34 62	Linseed- oil	17 lb.	About 1-7th.
								31 "	
Dec. 18, 1913	Aug. 10, 1914	235 days	4 out of 10	2843 2993 2840 3170	June 30 May 21 June 1 June 13	194 154 165 177	Beans	530 lb.	About 1-4th.
								380 "	
								410 "	
								460 "	
Dec. 18, 1913	Aug. 10, 1914	235 days	3 out of 10	2139 3166 2998	Jan. 6 May 30 May 20	19 163 153	Bran	47 lb.	About 1-6th.
								400 "	
								380 "	
Jan. 6, 1914	Aug. 10, 1914	216 days	3 out of 10	2874 2352 2987	Feb. 7 May 14 May 15	32 128 129	Mixed ration	110 lb.	About 1-5th.
								350 "	
								350 "	

Table VIII—(continued).

Experiment Com- menced.	Experiment Dis- continued.	Duration of Experiment.	Mortality from Lanzickte.	Number of Animals Concerned.	Date of Death, 1914.	Days of Feeding before Death.	KIND AND QUANTITY OF SUPPLE- MENTARY RATION EATEN.		
							Kind.	Approximate Total Quan- tity Eaten.	Computed Fraction of Maintenance.
June 13, 1914	Aug. 10, 1914	58 days	1 out of 10	3192	July 26	43	Crushed maize	130 lb.	About 1-3rd.
April 20, 1914	Aug. 10, 1914	112 days	2 out of 10	2383 2962	May 12 Aug. 6	22 108	Potatoes	300 lb. 1,500 "	More than 1-3rd.
April 21, 1914	Aug. 10, 1914	111 days	5 out of 11	2488 2603 2349 2615 2338	May 1 May 3 May 21 May 23 June 14	10 12 30 32 19	Pressed brewers' yeast	30 oz. 30 " 30 " 75 " 80 " 90 "	—
Jan. 6 and May 16, 1914	Aug. 10, 1914	216 or 99 days	4 out of 10	3137 2367 3134 2965	March 26 June 24 July 3 July 3	79 39 179 48	Rice	365 lb. 310 " 1,025 " 370 "	About half to complete maintenance.
Jan. 22, 1914	Aug. 10, 1914	200 days	4 out of 14	3148 2328 3151 2323	May 14 May 24 June 3 June 13	82 92 132 142	Controls (Grazing only)	—	Nil.

From this summary it will be seen that the incidence of lamziekte bears no discernible relation to the diet. The supplementary food-stuffs, ranging from one-seventh to one-half of maintenance requirements certainly effected marked improvement in the general condition of the cattle, but in no sense decreased the occurrence of the disease.

The diets supplying very considerable amounts of vitamine in the form of bran, beans, maize, and especially potatoes, effected no diminution whatsoever in the outbreak of lamziekte. On both the potato and the maize rations the number of cases would probably have been higher if the experimental periods had been as extended as that of, say, the treacle test. In the next section, comparing field and laboratory data, a sub-summary will be given, bringing out more specifically the vitamine contents of the supplementary stuffs.

Whether "condition," *per se*, has any influence upon the incidence of the disease is again very doubtful, although it is a farmer's wail that his best beasts go first. In these experiments, at least, the mortality amongst the controls left to their own resources on the veld is the same as that amongst the better-conditioned lot receiving beans. Taking the sum of all the rationed cattle, we have a mortality of 28 out of 82, or about 34 per cent., as against 4 out of 14, or 29 per cent. in the controls. This difference is entirely negligible, especially in consideration of the fact that animals added later to replace fatal cases are not taken into the percentages unless they themselves contracted lamziekte. In passing, it may be noted, however, that if mortality amongst controls be reckoned on the basis of extra animals on Armoedsvlakte in neighbouring camps or in other experiments, the figure is somewhat lower—about 20 per cent, instead of 29 per cent. The mortality amongst the rationed animals is therefore unusually high.

To these trials must be added the dietetic data of an experiment by Mr. W. H. Andrews, Veterinary Research Officer at Christiana, in which a heavy ration of Pretoria hay was fed. The ration in that experiment consisted of 56 lb. of maize, 56 lb. of bran, and 250 lb. of Pretoria hay for twenty-five cattle. Within a period of three and a half months three of these animals contracted lamziekte—a mortality of 12 per cent. This ration is interesting since it represents a mixed ration of $2\frac{1}{4}$ lb. of bran, $2\frac{1}{4}$ lb. of maize, and 10 lb. of Pretoria hay per head per day, in which, as we now believe, any one constituent contains enough of vitamine to ward off an avitaminosis.

Grass-feeding.—The grass growing in control Camp C at Armoedsvlakte was cut and fed to cattle at various times. The cattle were perpetually kraaled, so that for the natural grazing was therefore substituted a rough approach to it (or part of it at least), while at the same time factors other than the purely dietetic were largely eliminated. Grass cut at random and made into hay showed itself less efficient from a general nutritive point of view than the natural grazing, and to keep the cattle in condition a small variable supplementary ration was usually allowed.

Owing to the exigencies of contemporaneous work and the sparsity of grass on the veld, it was found difficult to keep a full batch of kraaled cattle feeding under conditions parallel to those of animals grazing naturally. The following summary, however, represents the test as carried out. Two cattle, Nos. 2485 and 2497, were kraaled continuously and fed from 15th February to 28th May, 1914, on

Camp C hay alone. On this latter date five more cattle, Nos. 3133, 2618, 2347, 2621, 2418 were added to the test and continued solely on Camp C hay until 10th August. From the same date (28th May) a further five animals, Nos. 2627, 3168, 2330, 2344, 2601, received the same *ad lib.* hay feeding, along with a condition allowance of $2\frac{1}{2}$ lb. per head per day, of the mixed ration previously mentioned. All twelve animals were now carried on from 10th August to 18th January, 1915, on Camp C hay, plus a small but variable condition allowance, when half the number (in poor condition) were used for another experiment, leaving Nos. 2627, 3168, 2347, 2497, 2621 still in the test and in good condition. These five have been continued on the lamziekte hay right up to the moment of writing (May, 1915).

Not one of the cattle contracted lamziekte, although two were in the experiment for 338 days, five for 235 days, and five for 307 days. Expressing it in another way, we may say that, between the dates of 15th February, 1914, and 31st March, 1915, 3386 "food-days" were registered by all the cattle. Taking all the cattle dying of lamziekte on Armoedsvlakte between these dates, and taking into account the length of time spent on the farm, we have a general mortality of about 22 per cent. In the twelve cattle on lamziekte hay, the expectation of death would therefore be about two cases, although, owing to the small number of cattle involved, chance might ordain five cases (Table XIII) or, as actually found, none at all. It is therefore not safe to assume that the factor causing lamziekte is something directly related to free veld grazing, although the evidence, such as it is, suggests such a view.

In the same connection, a similar experiment carried out under the supervision of Mr. J. Walker, Veterinary Research Officer, stationed at Grahamstown at the time, is of interest.

Ten cattle were fed at Grahamstown on hay cut at random from areas upon which twenty-three cattle naturally grazing over the lamziekte farm Nurney acted as controls. This hay was of extremely poor quality and a minimal supplementary ration of forage, mealie-meal, and bran, was given. The trial was continued for a year, during which time four of the controls contracted lamziekte but none of the experimental cattle—although five out of the ten ultimately died of poverty.

In another experiment carried out at the Onderstepoort Laboratory, lamziekte hay was obtained from the lamziekte farm Doornbult (Bechuanaland) and from another farm where a recent outbreak of the disease had carried off a number of cattle. Twelve cattle were fed *exclusively* on this hay from March, 1913, to October, 1913, when the supplies ran out. The animals sank into extremely low condition on the low-quality veld-hay, and when put out of the experiment some of them were on the verge of death through simple under-nutrition. *None*, however, contracted lamziekte.

In view of the survival periods in Table XIII (seventh column) these negative results cannot be explained away on the brevity of the feeding periods. Unfortunately, the additional hay-feeding experiments just described are not conclusive, but they at least increase the suspicion that an important factor in the causation of lamziekte is eliminated whenever the natural roaming over the veld is eliminated, and decidedly weaken any view which regards the disease as of purely dietetic origin.

The fact that hay made from grass cut at random from lamziekte areas should often prove so innutritious while the cattle grazing naturally over the areas themselves generally contrive to keep in fair condition, and to complete the cycle of their being in healthy fashion, except where they specifically contract lamziekte, is itself of some interest. It may be that the cattle under veld conditions make up a fair proportion of their diet from bushes excluded from the hay. It may be that, from the immense areas at disposal, they select the youngest and most nutritious grasses, whereas in the cut grass the tendency is for the longest and oldest "dead" grasses to preponderate.

Be that as it may, the fact remains that the cut fodder is generally (not invariably) of very low value, but, so far as can be judged from the limited nature of the experiments performed, shows no tendency to produce lamziekte. The cases in which a condition allowance was fed in addition to lamziekte hay do not, in view of Table XIII, militate in any way against the object of the test, which was not specifically related to the avitaminosis problem. The tests, however, in which lamziekte hay was fed as *sole* ration, are definitely of interest in relation to deficiency dietetics. The fodder, of lower value than the veld as a whole (as evidenced by the relative thrift in natural *vs.* kraal conditions), and therefore more likely to be vitamine-poor, did not produce any specifically recognizable deficiency disease. Instead, it resulted simply in the symptoms of delayed inanition or in death from what the farmer would call "poverty."

We are not disposed to discuss whether such poverty is in reality a chronic avitaminosis or not, i.e. whether the inanition symptoms arise from a specific deficiency in "minimum substance" and not from a general inadequacy of calorific or protein intake. We ourselves attribute the failure to thrive to innutrition in the latter gross sense, but even if it were after all due to a specific deficiency, it would go to support the view expressed in Section III, 2, that an avitaminosis in farm stock possibly shows itself as the generalised malnutrition of an atrophic form rather than as a clearly recognizable clinical entity. That "poverty" in *some* cases *may* be in reality a concealed deficiency phenomenon, due to a lack of some "minimum substance," either vitamine or amino-acid "Bausteine," or even mineral, is quite possible, but that involves separate research. The case of the Romney Marshes in England is reminiscent of such a possibility.

Periodic Incidence of Lamziekte.—It is not intended to discuss this question at any length here, but, at the same time, it is worthy of brief comment in relation to the experiments already described and in so far as it bears upon the vitamine theory.

From Table XIII we may re-arrange the mortality according to calendar months, at the same time summarizing the notes made by Mr. Sharpe upon the weather conditions of the veld. Animals commencing in the middle of a month are treated for convenience as from the beginning of the month. This is justifiable on the ground that in most cases the cattle were not actually drafted into experiment until some little time after arriving at Armoedsvlakte, and in any case the summary is rough and of very limited value owing to the small number (132) of animals considered. In the last column the fatal cases occurring amongst a variable number (say, 50 to 100) of cattle belonging to other experiments, but temporarily running in the same area as the dieting batches, are given.

Table XIV.

Month.	No. of Animals Considered.	Deaths from Lam-ziecte.	Per-centage Mor-tality.	LENGTH OF TIME ON ARMOEDS-VLAKE VELD.		Rain and Condition of Veld.	Additional Fatal Cases of Lamziecte amongst other Cattle on same Veld.
				No. of Cattle.	Months.		
Dec., 1913 (13 days only)	30	Nil	—	30	1	About $\frac{1}{2}$ " rain. Chiefly a shower on 14th, after which hot dry weather effected wilting of the growth stimulated by the shower	3 (whole month)
Jan., 1914	70	1	1 to 2	30 40	2 1	Nearly 3" of rain. From the first week the veld improved, giving fairly good grazing for the rest of the month	—
Feb., 1914	70	2	3	30 40	3 2	Weather at first hot and dry again and veld very poor. Improved greatly after the middle of the month. Total rainfall about 3"	—
Mar., 1914	70	2	2	30 40	4 3	Rainfall about 1". Grazing fair; weather cold at nights; towards end of the month veld drying up again	2
April, 1914	90	2	2	30 40 20	5 4 1	Rainfall about $\frac{3}{4}$ ". Most of grass in Camp "A" now exhausted. Veld dried up and wintry towards end of month	2
May, 1914	90	14	15	30 40 20	6 5 2	Scarcely any rain at all (0·1"). Frost at night. Cattle moved to Camp "B," where fair amount of green grass remains	6
June, 1914	100	7	7	30 40 20 10	7 6 3 1	Rainfall about 0·4", two slight showers about 12th, and 0·34" on 25th. Cold. Veld dry, but earlier herbage still abundant	3

Table XIV—(continued).

Month.	No. of Animals Considered.	Deaths from Lam-ziekte.	Per-centage Mor-tality.	LENGTH OF TIME ON ARMOEDS-VLAKTE VELD.		Rain and Condition of Veld.	Additional Fatal Cases of Lamziekte amongst other Cattle on same Veld.
				No. of Cattle.	Months.		
July, 1914	100	3	3	30	8	No rain at all. Veld very dry, but earlier herbage still abundant	4
				40	7		
				20	4		
				10	2		
Aug., 1914 (10 days only)	100	1	1	30	9	Scarcely any rain (0·1"). Extremes of temperature from frosts to hot winds. Veld very dry, but dry vegetation still plentiful	3 (whole month)
				40	8		
				20	5		
				10	3		
Whole pe-riod	132	32	24	—	—	8"—9" of rain	—

Very little can be deduced from the seasonal incidence of the disease as recorded in this table. It is noteworthy, however, that the proportion of cases was highest in May, and it is worthy of added comment that about this time lamziekte was reported as very prevalent in the district. No clear relation of prevalence to rainfall is brought out, although the opinion was expressed by some farmers in the district that fresh cases commonly occurred a day or two after a shower following a dry spell, and that the disease again abated after a heavy fall of rain.

The earlier observations that lamziekte is most prevalent in dry seasons still hold, but at the same time moderate rainfall involving marked improvement in the vegetation does not necessarily stop the disease, and cases have been observed to occur in rapidly growing green grass.

That the disease is not limited to dry seasons is indicated by certain earlier records—is shown for instance in relation to farms P.O. and L, Christiana District, in Mitchell's paper in the report already quoted (*l.c.* 1, p. 87).

The observations at Armoedsvlakte during the year 1912-1913 also show no clear relation of drought to prevalence of lamziekte. Since these observations cover the year preceding our experimental

period on the same area a summary (Table XV) of them may be inserted here:—

Table XV.

MONTH.	Rainfall in Inches.	Fatal Cases of Lamziekte.	MONTH.	Rainfall in Inches.	Fatal Cases of Lamziekte.
1912—June.....	0.15	None	1913—Mar.....	6.98	4
July.....	0.24	None	Apr.....	1.69	1
Aug.....	Nil	None	May.....	Nil	2
Sept.....	Nil	None	June.....	Nil	3
Oct.....	0.47	3	July.....	Nil	1
Nov.....	0.05	1	Aug.....	0.05	None
Dec.....	2.09	6	Sept.....	0.19	1
1913—Jan.....	1.57	8	Oct.....	1.68	1
Feb.....	4.93	12	Nov.....	0.75	1

During four months of practically rainless weather *no cases* occurred. Thereafter, with the exception of March, the mortality seems to be heaviest in the months with highest rainfall, when the grass is least open to suspicion of possible vitamine deficiency. Thus for November, after six months in which less than one inch of rain had fallen, we have only one case of the disease, whereas in December, after over two inches of rain we have six cases, and from January to March, during which time the rainfall was over thirteen inches, we have no fewer than twenty-four cases.

The comments on the weather in Table XIV link on to these records, and it may be also added that although in the months following our experimental period proper a relatively heavy rainfall was experienced, the occurrence of lamziekte by no means stopped. October, November, December, 1914, and January, 1915, each had a rainfall of over 2½ inches in the Vryburg District.

TREATMENT OF LAMZIEKTE WITH VITAMINE EXTRACTS.

Therapeutic treatment of the disease was attempted in various ways, but of these only the one involving administration of vitamine extracts need be mentioned. The product chiefly used was *orypan*, the extract from rice polishings already mentioned in Section III.

Animal 2182. Received 50 grammes orypan, equivalent approximately 0.15-0.2 grammes per kilo body-weight, intravenously in 500 c.c. of saline. No beneficial effect was observed, and the animal died of lamziekte two days later.

No. 2145. Showed signs of lamziekte on 18th March and received 50 grammes of orypan. The animal recovered, but in view of the natural recovery after mild attack observed in seven other animals (untreated) in the same experiment, and the negative character of subsequent tests, this recovery can hardly be regarded as other than spontaneous.

No. 3148. Received 100 grammes orally, equivalent to 0.3 grammes per kilo, but died on the following day.

No. 2987. Received 50 grammes orypan; died next day.

No. 2989. 100 grammes administered; died 3 days later.

No. 3166. Was treated with 400 grammes, half injected and half drenched. This represents a dose more than sufficient to cure the

body-weight of the animal expressed in pigeons, and the case was caught in the early stages of attack. From one thousand to two thousand pigeons could have been successfully cured of acute polyneuritis by this amount of orypan; nevertheless the cow died of lamziekte 4 days later.

No. 3151 was drenched with 150 grammes, and injected intrajugularly with 200 grammes. No beneficial results followed, and the animal died the same day.

No. 2695. 100 grammes of laevurine, a vitamine extract of French origin, but less curative than orypan, was injected intrajugularly in saline. The animals died within 3 days.

In addition to these cases several others were treated with varying amounts of orypan and laevurine, but the quantities administered were in general too small, and the results (all negative) were therefore discarded.

From these cases it is seen that vitamine extracts have no *therapeutic* value in the treatment of lamziekte. Since the dosages ranged from 0.15 gramme to 1.3 gramme per kilo body-weight, and a dose of 0.7 gramme per kilo was usually sufficient to cure polyneuritis gallinarum within a few hours, it cannot be argued that the doses for cattle were too small. On the contrary, considering that bovine metabolism is much more sluggish than avian, it is probable that the lowest dosage employed was more than adequate.

At the same time it is not possible to draw any definite conclusion concerning the nature of the disease from these cases alone. Theoretically it might be that in all cases of lamziekte the disease is too far advanced before the first symptoms show, and that vitamine treatment is ineffective for that reason. We do, however, interpret the negative nature of the results as strong collateral evidence supporting that of the feeding-tests with rations rich in vitamine, and affording further justification for our general conclusion that lamziekte is not an avitaminosis. This conclusion will be specifically drawn in Section VI, but before doing so a comparison between laboratory and field data may be made for the sake of more clearly bringing out the vitamine aspect of the question.

V. COMPARISON OF LABORATORY AND FIELD-DATA.

To some extent these data have already been compared incidentally, but for the present purpose it is instructive to compare directly the vitamine-content of the diets in the pigeon tests on bran, maize, potatoes, beans, and yeast, with that of the rations in the Armoedsvlakte trials where these materials were used in making up the supplementary feed of cattle on the veld. The tests with oil and treacle may be omitted, since the pigeon method failed to detect any antineuritic efficacy in these materials. The antineuritic efficiency of the veld-hay and oat-straw used in the rice-feeding trials could also not be determined, but it may be recalled that on so low an amount of coarse fodder as 1½ lb. per day (Table IX) in the rice diet of the laboratory cattle no avitaminosis was developed within a year, whereas lamziekte may be developed apparently within a few months on the exclusively coarse fodder ration of the veld.

For the purpose, however, of comparing diets in which the vitamine content is known to be fairly high we may summarize the results in Table XIII. The best basis of comparison seems to us to be the starch-equivalent standard. In this way the quantity of vitamine

supplied per food-unit metabolised is indicated irrespective of the absolute bovine or avian metabolism. The absolute gross weight of the supplementary ration may be expressed as a fraction of the calorific intake of the animal on the grounds that the vitamine requirements of an animal are probably proportional to its gross energy metabolism. In the case of pigeons the daily diet was equivalent to one-twentieth body-weight of rice. On the average this was 15 grammes, having a starch value of about 12 grammes (since rice is practically completely digested by pigeons). Where maize is protective supplement 4 grammes was found necessary to prevent polyneuritis, and the criterial fraction is therefore $\frac{1}{3}$ or 0.33. In the same way the starch-equivalent of the feed of cattle on the veld may be roughly assumed as 7 lb. per day, and the daily supplement of 3 lb. of maize therefore gives a comparative fraction of three-sevenths or 0.43.

We are not, it is needless to say, applying to cattle conclusions reached from experiments on pigeons (where the specific nitrogen-metabolism is so different), but are merely using the pigeons to test the diets in the same sense as they have been used in beriberi investigations on the human subject. In the nature of tests such as these, comparison can only be very rough, more especially as no account is taken of individual differences in vitamine requirements. Furthermore, the specific vitamine requirements of different classes of animals is an absolutely unknown factor, and the specific difference between the bird and the bovine may be enormous.

Table XVI.

PROTECTIVE FEEDING-STUFF CONSIDERED.	DAILY RATION PER HEAD.		DECIMAL EXPRESSING THE APPROXIMATE RATIO OF REAL WEIGHT OF FEEDING-STUFF TO TOTAL STARCH EQUIVALENT OF INTAKE.	
	Protective for Pigeons.	Supplied to Cattle.	Pigeons.	Armoedsvlakte Cattle.
	Grms.	lb.		
Bran.....	1.5	2.5	0.12	0.36
Maize.....	4	3	0.33	0.43
Beans.....	3	2.5	0.25	0.36
Mixed ration.....	2	3	0.17	0.43
Potatoes, raw.....	< 14	14	< 1.2	2.0
(Brewer's Yeast).....	2	(0.15-0.30)	0.17	(0.02-0.04)

From the last two columns it is apparent that with all the supplementary rations proper, the cattle dying of lamziekte (Table XIII) received an amount of vitamine *higher than that required by pigeons for the metabolism of same quantity of food* (starch equivalent). In the case of bran the cattle even received three times as much, and with potatoes about twice as much. In view of the probably very low vitamine requirements of cattle indicated by the laboratory rice-feeding experiments, the amount of vitamine actually supplied at Armoedsvlakte must be regarded as in *enormous excess of minimum* metabolic requirements.

With yeast, the quantity of vitamine supplied in proportion to food metabolised is much lower for the cattle than for the pigeons, and in this connection it may be explained that the object of the yeast trials was somewhat different to those involving the use of supplementary rations proper. As stated earlier, the natural concentrated foods, beans, maize, and bran, were given with the express purpose of covering the theoretically possible vitamine deficiency of veld pasture, for the reason that these materials are amongst those most commonly used both in this country and in Europe to supplement a hay ration for stock when natural grazing is unobtainable. It was therefore considered that they should contain the constituents which a poor hay or poor veld might be supposed to lack. For the sake of including a root moiety probably containing the more labile scurvy vitamine in place of the more stable beriberi vitamine of grain produce, a ration of potatoes was included in the experimental series. These two classes of protective stuffs were anticipated to yield the desired information relating to the avitaminosis hypothesis advanced for lamziekte, and the yeast tests were therefore arranged to test the points (a) whether the yeast therapeutic treatment of lamziekte in vogue in some districts, based upon the recommendations of Mr. Stead, had any real value; (b) in the unlikely event of lamziekte proving to be an avitaminosis after all, what quantity of yeast might be reasonably expected to be protective; (c) whether the cost of the prophylactic treatment, if effective, would be permissible from an economic point of view.

For this reason the quantities of yeast supplied were kept down to between one-tenth and one-fifth of the quantity calculated from pigeon tests, this quantity being gauged as probably still covering the requirements of cattle in which an avitaminosis, if it exists at all, is a matter of very slow development. In the light of the rice-feeding experiments at the laboratory, where the vitamine in the ration was vanishingly small, we feel that the yeast quantities given at Armoedsvlakte, although apparently low, were in reality liberal.

The desired information was at least obtained, and it can be definitely stated that the Stead treatment from a prophylactic, and therefore from a therapeutic, point of view, is valueless.

VI. IS LAMZIEKTE AN AVITAMINOSIS?

Considering the comments made in connection with the various preceding protocols, the only answer which can be offered to this question will be already obvious. In specifically expressing the conviction that there is no relation between lamziekte and vitamine deficiency it is well, however, to marshall all the evidence in one place.

(a) The cattle-feeding experiments at Onderstepoort have shown that cattle fed for periods of a full year upon a ration supplying a full calorie intake, and consisting of nine-tenths by weight of highly polished rice and one-tenth of poor hay or autoclaved oat-straw, *can* be maintained at their original weight and without showing symptoms of lamziekte. One of these cattle, a heifer, took the bull in the same experiment and successfully carried a calf to full term on the rice diet. Judged by accepted criteria, the diet upon which these cattle were fed was highly deficient and contained but little vitamine, an amount surely below that which an otherwise adequate natural grazing could be expected to show even under the worst conditions.

A proportion of fodder to rice, five times as high as that allowed to the cattle, had no marked effect in delaying the onset of polyneuritis in pigeons within the usual period of a month. Yet the cattle on an even more "vitamine-free" diet showed no avitaminosis within thirteen months.

Conclusion.—Either that the residual traces of vitamine in the diet were adequate for cattle, or that cattle are independent of an exogenous vitamine supply, or that the time required for the development of a theoretically possible avitaminosis is greater than a complete cycle of the seasons, and that therefore cattle which in practice do not survive in even passable condition through more than one year on exclusively drought pasture are not to be expected to show an avitaminosis in the field; that therefore the degree of probability that lamziekte is an avitaminosis is exceedingly low.

(b) In any experiments in which hay cut from lamziekte veld has been fed to cattle, lamziekte was not produced. Such hay represents part of the natural grazing, and from the fact that the cattle generally thrive very badly upon it, it may be assumed that it represents a part more likely to be "deficient" than the veld as a whole.

Conclusion.—That, in so far as the limited evidence in this direction goes, it points *against* the theory that lamziekte is an avitaminosis.

(c) The feeding of vitamine-rich supplementary rations to cattle on the lamziekte veld for long periods in no way diminished the prevalence of the disease, the mortality being (within the limits of error) actually higher than in the control cattle. The vitamine was supplied in the forms occurring in seed products such as maize, beans, and bran, in roots such as potatoes, and in yeast. The quantities so supplied were probably in enormous excess of the quantities theoretically requisite, and in some cases were as high as three times the amount required by pigeons (sensitive subjects) in metabolising the same number of food-units.

Conclusion.—That lamziekte is not an avitaminosis of any recognized type.

(d) The feeding of polished rice in quantity sufficient to cover from half to two-thirds of the metabolic requirements of cattle on lamziekte areas in no way increased the mortality.

We should therefore argue that polished rice contains as much vitamine as the natural herbage, or that lamziekte is not an avitaminosis. If, on the other hand, the failure to develop lamziekte or any other supposed avitaminosis in the rice-feeding laboratory experiments be attributed to the traces of vitamine still present in highly polished rice, then the same traces should have prevented lamziekte in the Armoedsvlakte cattle if lamziekte were an avitaminosis.

(e) In regard to the treacle and oil rations, we may consider, not the materials themselves, but the small quantity of Pretoria hay upon which they were smeared for convenience in feeding.

This hay in quality was the same as, and in quantity rather higher than, that given to the rice-feeding cattle at the laboratory, so that if the failure to develop an avitaminosis in the latter case be attributed to the vitamine in the small ration of coarse fodder, the same reasoning should hold for the Armoedsvlakte cattle, and we would again have to conclude that lamziekte is not an avitaminosis.

Whatever protective influence is possibly exerted by hay is however ruled out by the Christiana experiments, in which 10 lb. per head per day was given.

(f) Therapeutic treatment with vitamine extracts had no effect. This supports the conclusion deduced from the feeding of supplementary rations.

(g) To these inductive and deductive arguments may be added those baseable upon teleological grounds and supported by the evidence collected from lamziekte areas in general. The argument emphasized by Theiler (*l.c.* 1) that cattle which *thrive* on a lamziekte veld in the sense that they maintain condition and continue growth are not likely to do so on a diet deficient in any vital respect, is a strong one. Furthermore, it has behind it an experimental support in the fact that the development of any known avitaminosis is usually accompanied by a general falling off in condition and drop in body-weight; often also by partial cessation of growth. In the atrophic form of deficiency diseases, emaciation and loss of weight is extremely marked. In the dropsical form the emaciation is concealed by water-logging of the tissues, but clinical and post-mortem examination of lamziekte definitely excludes dropsical condition—even “slight oedema” is rare.

Growth in young cattle, it is true, is slow on these areas, but no slower than on similar veld free from lamziekte. The growth, however, is not stunted, and cannot be said to be regularly retarded. The slow growth is attributable rather to breed than to diet in so far as Bechuanaland, at least, is concerned, and the appearance and generally excellent condition of the animals leaves the European observer of the veld at a loss to understand where the food comes from.

Supporting the evidence of the supplementary ration tests at Armoedsvlakte, there is the fact that herbage on lamziekte farms is often as good as vegetation on other poor farms *free* from lamziekte, and there is the observation of a fair number of cases in which lamziekte was diagnosed by professional veterinarians as occurring on areas covered with an almost luxuriant (relatively) green vegetation after preceding rains. Surely the change from sparse drought vegetation to comparatively abundant young green pasture should effect cessation in outbreak of an avitaminosis. Such observations, however infrequent, appear to us to be capable of far-reaching interpretation.

Taking all the evidence into consideration, we have no hesitation in expressing the conviction that *lamziekte is not an avitaminosis*. We at least see no way of squaring the experimental evidence with the supposition.

Appendix I.

DOES AN AVITAMINOSIS EXIST IN FARM STOCK AT ALL?

To this question it is of course impossible to give an answer, but it forms a useful starting-point from which to consider a few papers which have recently appeared and in which deficiency theories have been advanced for certain diseases in other parts of the world. *A priori*, there is no reason for believing that a class of bodies, the vitamins, known to be indispensable for proper nutrition and growth in man, in mice, in birds, in guinea pigs, and in other animals, and

constituting an essential constituent of their diet, should be exogenously unnecessary for cattle, horses, pigs, sheep, and goats.

It is not justifiable to assume without very definite evidence that these animals are capable of directly synthesising vitamins for their own requirements. Funk (*l.c.* 3, p. 59) has suggested that the *form* in which an avitaminosis manifests itself may perhaps be related to the type of purin metabolism of the animal concerned, and that animals in which a uriko-oxydase is absent (man, birds) can show the beriberi type of disease, whereas those in which the urikolytic enzyme is present (probably most other animals) only show deficiency symptoms of the scurvy type. Either way, however, there is no reason to assume that any animal is immune from some sort of an avitaminosis, and all we wish to bring out is that in farm stock it is very difficult to produce. In our experiments no evidence of such could be obtained even after feeding highly deficient diets for prolonged periods. This holds more particularly for cattle where the periods were longest, and, as already stated, we think it highly improbable that an avitaminosis will ever be detected under practical conditions of cattle-rearing even in the worst seasons. We rather believe that on a poor natural drought pasture, death from inanition or "poverty" would ensue before a specific clinically recognizable avitaminosis could manifest itself, and, incidentally, think it at least possible that the vitamin requirements of cattle are so low that they may even be covered indirectly by synthesis carried out by the extensive bacterial flora of the intestines. We are not of course prepared to exclude the bare possibility that *some* forms of "poverty" may in reality be a concealed atrophic avitaminosis, but, meantime, do not think such a speculation justified.

From New Zealand has been reported a cattle disease termed "bush-sickness," investigated chiefly by B. C. Aston (Government Chemist) and C. J. Reakes¹⁵ (Government Veterinarian).

It has been described by Gilruth as a "progressive anaemia," and various deficiency theories have been advanced for its causation, although emphasis is laid (by Aston) rather on the idea of a mineral deficiency than of vitamin-hunger. The disease cannot be said to resemble lamziekte in any way, but is of interest in relation to the subject of dietetic deficiency in general. A disease of sheep, "Osseous cachexia," is also reported by Reid and Aston¹⁶ and attributed to mineral deficiency, but as we hope to consider this more fully in connection with later work on skeletal diseases and mineral metabolism of stock, further discussion may be omitted at present.

A number of diseases of stock going under vague names such as "dry bible," "coasting," "midland disease," "enzootic paraplegia," and incidentally also "bush-sickness," are all hinted at as conceivable avitaminoses, by Place, in a lecture delivered before the veterinary section of the Australasian Association for the Advancement of Science, and reprinted in various papers, including the *Journal of Agriculture of S. Australia*, and, in this country, the *Farmers' Weekly*.¹⁷

Place's paper, however, is popular in style and of a general speculative nature, neither aiming at bringing experimental evidence

¹⁵ *Journal of the New Zealand Department of Agriculture*, Nov., 1911, Aug., 1912, April, 1913, Feb., 1914.

¹⁶ *Journal of the New Zealand Department of Agriculture*, 15th Nov., 1910.

¹⁷ *Farmers' Weekly*, Vol. V, No. 125, 1913.

in support of the speculative ideas nor at making any clinical or pathological analysis of the diseases referred to, but rather at pointing the need for research in the direction of veterinary dietetics.

Of greater direct value is the interesting paper by Henry¹⁸ on a new disease in the Bega dairying district of New South Wales. Henry, in the course of veterinary health inspection in the district, had occasion to observe an unknown complaint in cattle, first reported in 1912, and records his observations of the two ensuing years. He draws an analogy between the disease of the Bega district, the "impaction paralysis" of South Australia, and the lamziekte of this country, and regards it as markedly associated with soil deficiency in lime and phosphate and consequent food deficiency in phosphates. He finds that the disease occurs at the end of droughty periods in areas much depreciated by rabbit infestation, and that mortality areas coincide with those upon which "bone-chewing" and osteomalacia have been present for some time. Henry considers that great benefit is obtained by feeding small rations of nutritious food, and especially by returning skim milk to the cows. Evidence of infection or intoxication is all negative, and, as just stated, a phosphate deficiency theory is advanced to explain the causation of the disease.

Even taking "phosphate deficiency" in its widest sense as meaning not merely deficiency of phosphate as such, but any deficiency of which the phosphorous content in a foodstuff may be the index, we are inclined to regard Henry's view as rather belonging to the category of the earlier deficiency theories of Hutcheon for lamziekte, Ingle for osteoporosis, and Schaumann and others for beriberi and scurvy. At one time lamziekte was thought to be associated with soil deficiency in lime and phosphate, but this generalization has been upset by identification of the disease on all sorts of soils, and its absence on so many soils definitely similar to those of typical lamziekte farms.

Incidentally we seriously doubt whether ordinary determination of the phosphorous content of grasses offers any indication of a deficiency which could result in a specific deficiency disease. The argument in the classical work of Frazer & Stanton,¹⁹ in which the phosphorous content of polished rice was held to be a practical index of the fitness for consumption as *sole* diet, cannot be safely applied to other products. The lowered phosphorous content of polished rice is, of course, merely an index of the degree of polishing, or removal of the subpericarpal layers, and it has *yet to be shown* that any direct relation subsists between phosphoric acid and "minimum substances" in natural untreated foods. We are ourselves at present investigating this point.

A direct experiment on the value of supplementary rations, limited to a single camp and eliminating the element of varying date but retaining the factor of constant natural grazing, would be of great interest in connection with the Bega disease. As previously mentioned, lamziekte was in many quarters held to be preventable by the use of supplementary rations, but the direct experiments described in this paper contradict that view, and it is *possible* that the observations made so far by Henry on the effect of returning the skim milk to the cows, or feeding small quantities of maize, are attributable to coincidence.

¹⁸ Max Henry. "Mortality of Cattle in the Bega District of New South Wales." *The Veterinary Journ.*, Feb., 1915, Vol. 71, No. 476.

¹⁹ Frazer and Stanton. "The Etiology of Beriberi." *Studies from the Institute of Medical Research, Federated Malay States*, No. 12, 1911, p. 85.

Appendix II.

EXPERIMENTS OF MR. STEAD.

In regard to Mr. Stead's evidence in favour of the vitamine-deficiency theory, little need now be said, more especially as Mr. Stead, after private communication of our experimental evidence, has more or less come round to our point of view. At the same time it is advisable to devote brief consideration to the experiments actually published²⁰ since they have been taken up at more than their face value by investigators in other parts of the world, and a tendency is shown to base hypotheses for other unknown diseases, presenting certain features of similarity to lamziekte, upon the assumption that lamziekte is an avitaminosis.

Stead fed one batch of thirteen pigeons on polished rice plus 4 grammes each of dried lamziekte flesh, and another batch on rice plus 4 grammes of dried healthy karroo flesh. The first lot developed nine cases of polyneuritis, the second lot only one. From this experiment Stead concluded that lamziekte flesh was deficient in vitamine as a result of depletion upon a deficient diet during life.

Contemporaneously with our own cattle-feeding experiments Stead also obtained cattle through the Division of Veterinary Research, fed them for a few weeks on oat forage to improve their initially low condition, and then fed different batches of five on diets of polished rice, samp, and white flour, plus a small (unspecified) ration of oat forage in each case. After the lapse of from ten days to a few months symptoms of depraved appetite, stiffness, and pain in walking, were observed in most of his animals. On this evidence Stead makes the comment: "There is not the least doubt that the symptoms noted are due to the polished rice diet, but the condition is certainly not lamziekte, neither is it stijfziekte; but it is as closely related to either of these as one is to the other."

These experiments of Stead are interesting in themselves, but from our point of view have no real bearing upon the problem of dietetic deficiency, and his deduction, "*putting the results obtained with cattle and pigeons together it is evident that there is a deficiency of beriberi vitamine in lamziekte cattle . . . that a deficiency of beriberi vitamine coexists with lamziekte and lamziekte veld,*" is surely unwarranted.

The fact that cattle reared on areas under conditions of relatively intensive agriculture and receiving supplementary rations of concentrated or succulent foods, obtain more vitamine in their food than do cattle reared under ranching conditions on the veld, is *a priori* acceptable and requires no proof. At the same time the fact has little bearing upon the question of minimum vitamine requirements for cattle, since the real question is not whether the veld is or is not vitamine-poor, but whether it is or is not deficient.

In regard to Stead's pigeon experiments the following points are worthy of consideration:—

(a) Only 4 grammes of dried flesh were fed. This quantity is, as a rule, insufficient even if a definitely protective sample of normal dried flesh be used. Cooper's minimum quantity was 5 grammes, and in our own experiments [III (1)] still higher quantities of normal biltong were often required to protect from polyneuritis.

²⁰ Stead. *Agric. Journ. of Union of South Africa*. May, 1914, p. 725.
do. do. do. July, 1914, p. 90.

We also found the variations of different samples of normal flesh *inter se* sometimes as great as the difference between lamziekte and normal flesh. No conclusions can therefore be drawn, since the experiment itself is not free from objection. Incidentally Stead's pigeons were not force-fed—if they had been it is probable that the whole lot, controls as well, would have gone down.

(b) A constant difference of vitamine content in dried flesh from ill-fed cattle from one area and well-fed cattle from another *might* conceivably be detected by the pigeon method (in spite of the big margin of error which must be allowed in such "biological analysis") and yet bear no *causal* relation to a real avitaminosis. Some depletion in vitamine reserves might conceivably occur in many diseases as an incidental concomitant in the pathological sequelae. An observed difference in vitamine content might also be idiosyncratic, as in the case of the muscular content in purins (or their mother-substances) or in glycogen, where adventitious variations can occur within considerable limits, both in respect to different tissues of the same animal and similar tissues of different animals.

Some falling off in antineuritic efficiency of flesh may possibly occur during post-mortem changes, drying out, and prolonged storage.

Finally, in further consideration of the fact that death from definite polyneuritis (and presumably from other avitaminoses) does not involve a complete robbing of tissue vitamine reserves [III (1)], we consider that no conclusions could be drawn from minor differences in the vitamine content of normal and lamziekte flesh, even if such were definitely established.

In relation to Stead's cattle-feeding experiments the following points are to be noted:—

(a) From private information communicated by Mr. Stead himself, the quantity of oat-forage (still containing a fair proportion of full ears), though not large, was many times greater than the quantity of veld hay or autoclaved straw fed in our experiments. Indeed, we seriously doubt whether Stead's diet can be really regarded as vitamine-deficient at all (for cattle), and from our point of view might justly be ruled out of court on this account alone.

(b) The symptoms observed do not warrant the assumption of dietetic deficiency. Similar symptoms may often be observed after a sudden change from coarse fodder to concentrated foods *rich* in vitamine. The symptoms in some cases also manifested themselves in so short a time as ten days—which fact speaks decidedly against an avitaminosis, even on Stead's own assumption that the cattle were already depleted on the previous vitamine-poor herbage of the veld. In our own cattle [III (2)] the same symptoms were manifested at various times, more particularly during passing attacks of digestive disorder (gastro-enteritis and diarrhœa), but the cattle recovered again, either spontaneously or on treatment with bismuth, and were carried on to the expiration of periods up to a year without showing any symptoms which could be fairly diagnosed as an avitaminosis.

We interpret Stead's "deficiency symptoms," therefore, as a transient, recurrent, dietetic laminitis which could have occurred even if the rice had not been polished and so devitaminized.

As just mentioned, however, Mr. Stead discontinued his experiments after comparing notes privately with us, and we have only recorded our differences for the reason already given.

VII. SUMMARY AND CONCLUSIONS.

The primary economic purpose of the investigations detailed in the text was to obtain evidence for or against the hypothesis that the South African disease known as lamziekte or gal-lamziekte, a neuro-muscular fatal complaint of cattle, is an avitaminosis. Correlated to this was an attempt to produce an avitaminosis in various classes of animals and to obtain data of general interest in scientific dietetics. The evidence incidentally acquired suggesting other theories of causation, is not specifically considered.

Pigeon-feeding tests were carried out to determine the anti-neuritic properties of various foodstuffs, more particularly those fed as supplementary rations to cattle naturally grazing over lamziekte veld suspected of vitamine-deficiency.

On the basis of these tests, different batches of cattle on the affected area were supplied with vitamine in the form of beans, bran, maize, potatoes, and yeast, in amount in large excess of the theoretical quantity required for the metabolism of the gross food eaten. At the same time another batch was supplied with a liberal ration of polished rice with the idea of still further reducing the vitamine-content of the natural diet. The mortality in the batches receiving supplementary vitamine-rich rations and in the batch receiving polished rice, was the same as that of the control animals, and the conclusion was therefore drawn that the prevalence of lamziekte bears no relation to the vitamine-content of the natural pasturage.

Kraaled cattle fed on hay cut at random from lamziekte areas did not contract the disease, and in so far as the limited nature of the experiment allows of a conclusion, the disease would appear to be non-dietetic (*in the nutritional sense*) in origin.

No clear relation of rainfall to prevalence of the disease on the experimental area could be detected.

No clear difference in mortality could be detected amongst cattle in good condition and those in poor condition. An average mortality of about 30 per cent. in the experimental cattle prevailed independently of feeding or condition.

Therapeutic treatment with vitamine extracts, even in theoretically excessive amounts, and prophylactic treatment with yeast, proved useless.

Cattle fed for periods up to thirteen lunar months on synthetic rations of exceedingly low vitamine-content, as judged by accepted criteria and pigeon analysis, failed to develop either lamziekte or any specific disease which could be diagnosed as an avitaminosis. This is regarded as strong corroborative evidence against the vitamine-hunger theory as applied to lamziekte, and the view is incidentally expressed that an avitaminosis in cattle is not likely to occur in practice unless manifested simply in an atrophic form clinically indistinguishable from inanition.

Horses, fed upon diets similar to the polished rice rations fed to cattle, also developed no symptoms of specific deficiency disease within periods of six months, and the conclusion is drawn that an avitaminosis in the horse, if it exists at all, is a matter of slow development, and would tend rather to be obscured by preceding inanition, on any naturally occurring diet upon which the animal could conceivably be fed.

Dogs fed on polished rice succumbed, after periods varying up to four months, under symptoms suggestive of generalized malnutri-

tion rather than those of a specific clinically recognizable avitaminosis, of either the beriberi or scurvy type. Specific rather than general deficiency is, however, regarded as the cause of death, although the calorific intake was not sufficiently accurately ascertainable to settle the point.

Lamziekte biltong showed itself probably as efficient as ordinary biltong in enabling dogs to maintain equilibrium on a polished rice diet, and in restoring dogs, emaciated after rice feeding, to normal health. These experiments are regarded as analagous to pigeon tests in which no marked constant difference in vitamine content could be detected between dried flesh from healthy animals and that from diseased animals. The dogs showed no ill-effects as a result of eating the diseased biltong.

Pigs, goats, and sheep, fed for prolonged periods on rations in which polished rice was either the exclusive or the preponderating constituent, did not develop any specific deficiency disease. No definite conclusion, however, is drawn from these experiments, since the protocols are open to interpretation in more than one way.

A number of points in connection with the problems of growth and general dietetics are raised.

SHEEP SCAB.

**Observations on the Life-history of *Psoroptes communis*,
var. *ovis*, and some points connected with the epizootiology
of the disease in South Africa.**

BY

A. W. SHILSTON,

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ALTHOUGH sheep scab has for many years been a scheduled disease in South Africa the efforts made to bring about its eradication have in the past met only with limited success. To a large extent this must be attributed to limitations in the matter of efficient control incidental to the area and character of the country and the diverse nature of its inhabitants.

There are, however, certain features of the disease which the generally accepted account of the life of the causal parasite fails to explain, and until exact information on these points is available the result of repressive measures must be very uncertain.

Reference to the literature of sheep scab shows that for practically the whole of our knowledge of the life-history of the acarus we are indebted to investigators who recorded their observations over fifty years ago; of these the chief are Hering (1835), Hertwig (1835), Delafond and Bourguignon (1854), and Gerlach (1857).

Later writers of the disease have to a large extent been content to quote the statements of these authorities, especially those of Gerlach, and with the exception of observations published by Stockman while the present investigation was in progress, little attempt appears to have been made to ascertain whether the parasites exhibit similar biological characters in countries differing widely in climatic conditions from those in which the original observations were made.

This is certainly true of South Africa. Statements have been made regarding the vitality of acari or their eggs apart from the sheep, but the evidence brought forward in their support does not warrant the conclusions which are drawn.

The observations recorded here were made with the view of obtaining more exact information on the development of the scab parasites on the sheep and of determining the correctness or otherwise of certain popular beliefs regarding the disease; also, if possible, discovering the true explanation of the occurrences which have given rise to these beliefs.

The subject will be dealt with under the following headings:—

- (a) The life-history of the *Psoroptes communis*, var. *ovis*, on the sheep.
- (b) The interval to be allowed between dippings.
- (c) The duration of vitality of acari and their eggs apart from sheep; infectivity of kraals, etc.
- (d) The possibility of the *Psoroptes communis*, var. *ovis*, maintaining its existence on animals other than sheep or of the psoroptes of such animals producing scab in sheep.
- (e) Variations in the rapidity of multiplication of the acari.

(a) THE LIFE-HISTORY OF THE SHEEP SCAB ACARUS.

Observations made on the Sheep.

In order to observe the various phases of development through which the psoropte passes on the sheep, beginning with the egg and ending with the laying female, it was found necessary to provide a means of confining the parasites to a particular spot on the sheep where they could be watched unobscured by wool or crusts, but under as natural conditions as possible in regard to feeding and ventilation.

After several devices had been tried, the following method was adopted and has been found to give very satisfactory results: A number of covers were made by cutting rings 3 inches wide from a glass tube 2 inches in diameter and $\frac{1}{8}$ inch in thickness; over one end of these rings a double layer of fine butter muslin was stretched and fixed with seccotine and thread, thus forming a miniature tambourine. The free edge of each ring was ground smooth and rounded to prevent it cutting the sheep's skin. To maintain a cover in position on a sheep's back, the wool was clipped from a circular patch the same size as the ring and the skin carefully shaved; on this area the parasites or their eggs were placed, the cover, numbered for identification purposes, applied and melted paraffin wax run round it; this last kept the cover in position and prevented escape of the acari. The surrounding wool was then gathered up and tied sufficiently firmly to prevent the ring from slipping, but not so tightly as to completely exclude air from the cover. The muslin top was found necessary for the escape of moisture from the skin which otherwise condensed causing softening of the epithelium, suppuration, and the death of the acari.

Every three or four days the parasites were moved to a fresh patch as the growth of the wool and the formation of crusts soon made it difficult to observe them clearly.

While under observation the parasites were frequently removed from the skin and examined under the microscope to note the changes from one stage to another, but this treatment appeared to have little effect in retarding their development.

Seasonal variation of temperature is usually believed to have considerable effect in shortening or lengthening the duration of the life-cycle of the *Psoric acari*, and in many species of animal this influence is probably considerable. The sheep, however, has a very efficient covering during the winter months, and it is doubtful whether the temperature of its skin is then much lower than in summer when its fleece is short. At all events the observations made in Natal during the past two years have shown that there is little difference in the length of time required for the complete life-cycle of the acarus at the different seasons of the year, provided alteration of the temperature of the air is the only variant factor present.

Life-History.

To obtain eggs of which the time of laying was known fairly exactly, numbers of adult female acari were placed under covers on a sheep and twelve hours later the eggs were removed and transferred to a freshly prepared patch or used for observations *in vitro*.

When in close contact with the sheep's skin the majority of the eggs hatch after an interval of two days from the time of laying, and usually no eggs remain unbatched at the end of three days. They

have never been observed to hatch in less than two days. Eggs on the surface of thick crusts may not hatch for four or five days, but usually the eggs are deposited at the outside edge of the patch of affected skin where scab formation has not commenced.

To ascertain how long eggs might remain in the wool before hatching, a number of these were tied in a lock on a sheep's back, being separated about 2 inches from the skin; larvae began to come out on the sixth day, and by the ninth all the eggs had hatched. The weather at the time was very warm, the temperature varying from a minimum of 60° F. to a maximum of 100° F. in the shade, and as the observations on eggs *in vitro* recorded later showed it was quite possible for hatching to occur without any additional source of heat being necessary. It is probable that in cold weather eggs in the fleece at a little distance from the skin would not hatch in that situation, and would only retain the power of doing so for the same length of time as those kept entirely apart from the sheep. The greatest length of time that eggs may remain on the sheep and still be capable of hatching is of importance in determining the interval to be allowed between dippings, but as it is included in the much more important question of duration of vitality of eggs apart from their host, the observations will be recorded later in that direction.

As in all the acarina the larva is six-legged (hexapod), the two anterior pairs only being furnished with suckers, the posterior pair carrying two long bristles each. On hatching the mites very quickly attack the skin, a minute vesicle, containing yellow serous fluid, marking the situation of each puncture. During the first twenty-four hours they enlarge rapidly, but the frequent moultings said to occur at this stage to allow of enlargement have not been observed.

It may here be remarked that complete shedding of the skin has only been seen to occur as a necessary accompaniment of change from one stage of development to another, and it is always preceded by a resting period of considerable duration.

The process of moulting is not merely a casting off of the old skin, but is a fresh embryonic development inside the animal body similar to that occurring in the impregnated ovum. All the internal tissues undergo liquefaction, with the exception of certain germinal cells, and from the rapid multiplication of these a new form arises which presently breaks through the old covering in the same way that the larva emerges from the egg.

While these changes are taking place the acari remain perfectly quiescent, the length of time they occupy varying from about twelve hours, in the case of larvae moulting to nymphae, to nearly thirty-six hours in the later stages.

The larval stage is short, nymphae usually appear forty-eight hours from the hatching of the eggs and always within three days.

Nymphae may easily be distinguished from larvae by the presence of a fourth pair of legs, but when fully engorged they closely resemble the adult pubescent females.

They may be identified by the presence of slender suckers of the fourth pair of legs, and the absence of copulatory tubercles at their posterior extremity.

The nymphal stage lasts from three to four days, and during the first two days increase in size is rapid, while the period of quiescence preceeding moulting is usually about thirty-six hours. At this time the sex of the next stage can frequently be determined by the size of

the resting nymphs, the smallest usually giving rise to males. It has also been noticed that pubescent females always appear before males; the former have been observed five and a half days after the hatching of the eggs, but the latter have never been seen earlier than the sixth day.

The pubescent female possesses two copulatory tubercles, one on each side of the cloaca at the posterior margin of the body; the third and fourth pairs of legs are furnished with two long bristles each in place of suckers.

The male acari are readily recognized by their smaller size, the long third pair of legs bearing suckers and a small fourth pair without; also by the presence of the posterior tubercles and copulatory suckers and the brown plates on the posterior half of the dorsal surface of their bodies. The males are much more active than the females.

The proportion of males to females was stated by Gerlach to be as one is to two; whilst this has been noticed in some cases, the females are more often three or occasionally four times as numerous as the males.

The acari feed for a short time after moulting and then copulation takes place; this has been observed just over six days from the time of hatching. The pair usually remain united for about twenty-four hours, though when the females greatly exceed the males in number a shorter time may be occupied. Frequently the male remains attached to the female for over two days or until the latter moults. This she usually does two days after the commencement of the attachment of the male.

The now ovigerous female is distinguished by the presence of a sub-thoracic valva and suckers on the fourth pair of legs; both the third and fourth pair of legs are longer and stouter than in the pubescent female, while the copulatory suckers have disappeared. After feeding the ovigerous female is considerably larger than any of the other forms, and, like the male, its body becomes quite opaque, losing the semi-transparent appearance of the younger stages.

The first egg may be laid twenty-four hours after the last moulting, or nine days from the time of hatching of the larva from the egg.

In one observation two male acari were placed under a cover with numerous nymphs, of which the time of hatching was known. The pubescent females moulting out before the males of the same age, mated with the two older males, and the first egg was found eight and a half days from the time the original eggs hatched on the patch.

Even in mid-winter the complete life-cycle, as observed under a cover, never occupied more than ten days, and in the great majority of cases occurred in little over nine days. In summer eggs were constantly found nine days from the time of hatching of the original eggs.

It should be stated that sheep with heavy fleeces were always used to enable the glasses to be kept in position, thus the temperature of their skin may have been maintained at a higher point during the cold weather than would have been the case in sheep less well clothed.

Gerlach, who carried out his observations in 1857, but who is still almost the only authority quoted on the life-history of the sheep scab parasite, stated that the time required for a generation of acari to reach maturity was fourteen to fifteen days.

Other writers mention twelve to sixteen days, but there is a great lack of certainty and wide variation in the statements of the length of time required for the hatching of the egg and the duration of the various stages.

The observations here recorded have been repeated at intervals over a period of nearly two years, and they clearly show that so far as Natal is concerned the *Psoroptes communis*, var. *ovis*, can mature much more rapidly than records of observations made in other countries have led us to suppose.

The complete life-cycle may be briefly illustrated by giving the details of an observation made towards the close of the winter season:—

3rd August, 1912.—A number of acari eggs, laid during previous night, placed under cover on sheep.

4th August (1st day).—No eggs hatched.

5th August (2 days).—Several larvae present.

6th August (3 days).—No unhatched eggs observed, larvae increasing in size.

7th August (4 days).—Two or three nymphae present.

8th August (5 days).—All nymphae (enlarging).

9th August (6 days).—Nymphae enlarging, several quiescent.

10th August (7 days).—Some pubescent females present, no males observed, a few nymphae not yet moulted.

11th August (8 days).—Two pairs in copula, numerous females; all transferred to fresh spot.

12th August (9 days).—Four pairs in copula, numerous females.

13th August (10 days).—Four pairs in copula, apparently different females, as several others are enlarging and ready to undergo last moult.

14th August (11 days).—Numerous ovigerous females. Three eggs found nine days from time of hatching of original eggs.

Although each male acarus usually mates with from two to four females, it is apparently capable of fertilizing more than this number; in one observation six females were fertilized by a single male.

The females continue to lay eggs for a considerable time, and since the observations carried out to determine the number of eggs laid by each female also furnish information as to the duration of their lives, these will now be described.

Here again the majority of writers are content to quote Gerlach's estimation of fifteen to twenty-four eggs from each female acarus, overlooking the fact that this was made from the *Sarcoptes scabiei*, var. *hominis*, and is hardly applicable to the psoropte of the sheep.

There are two difficulties to be overcome before an actual enumeration of the eggs laid by a given female can be made.

Firstly, the acarus must be allowed to feed during the whole of the laying period. A considerable portion of the ovigerous females removed from their host will lay one or perhaps two eggs each, provided the temperature is not much below that of the body, but without feeding no further eggs will be laid; the only reliable method, therefore, of obtaining accurate information is to count the eggs actually laid by one or more females on the sheep itself.

The second difficulty arises from the fact that the period of egg-laying exceeds that necessary for the complete development of the acari, so that the second generation of parasites will mature and begin to lay eggs before the first generation has ceased to do so. By

examining the patches and removing the eggs each day this possibility was avoided.

It was also found necessary to transfer the females to a freshly prepared area of skin every three or four days owing to the growing wool and crusts obscuring the eggs. Even then, eggs occasionally escaped detection until they hatched, but when this occurred the larvae found were recorded as eggs laid two days previously.

When the females were removed, the old patch was examined for a further three days to ascertain whether any larvae appeared from eggs that had been overlooked.

The following observations are recorded in full, as they show not only the numbers of eggs laid, but also the rate and duration of oviposition and the total life of the female acari:—

First Observation.

Five pairs of acari in copula, placed on clean sheep on 29th January, 1913.

Date.	Age of Acari in days.	No. of Females laying.	No. of Eggs laid.	Remarks.
31/1/13.....	8	—	—	Male acari removed.
1/2/13.....	9	5	—	Patch not examined.
2/2/13.....	10	5	—	Patch not examined.
3/2/13.....	11	5	15	1 female missing, remaining 4 moved to fresh patch.
4/2/13.....	12	4	9	1 female dead.
5/2/13.....	13	3	12	
6/2/13.....	14	3	21	
7/2/13.....	15	3	23	
8/2/13.....	16	3	14	
9/2/13.....	17	3	6	
10/2/13.....	18	3	20	
11/2/13.....	19	3	19	
12/2/13.....	20	3	10	1 female killed, crushed under edge of cover.
13/2/13.....	21	2	11	
14/2/13.....	22	2	9	
15/2/13.....	23	2	4	
16/2/13.....	24	2	8	
17/2/13.....	25	2	11	
18/2/13.....	26	2	11	
19/2/13.....	27	2	9	
20/2/13.....	28	2	9	
21/2/13.....	29	2	4	
22/2/13.....	30	2	18	
23/2/13.....	31	2	6	1 female dead.
24/2/13.....	32	1	6	
25/2/13.....	33	1	6	
26/2/13.....	34	1	2	
27/2/13.....	35	1	0	
28/2/13.....	36	1	1	
1/3/13.....	37	1	0	
2/3/13.....	38	1	3	
3/3/13.....	39	1	0	Female dead.
		Total...	267	

It will be seen that one female can only have contributed to the first fifteen eggs laid, though missing when the count was made,

while another was dead at the second enumeration. It will therefore be fair if we assume that they only laid their share of these eggs, namely, six of the fifteen found on 3rd February, 1913, and, say, three of the count of the 4th.

If these nine eggs are deducted from the total collected, the remaining 258 were laid by three females, or an average of 86 eggs each. Since one of the three was killed whilst still actively laying, it is not improbable that the two remaining females laid nearly 100 eggs each.

The first eggs were probably laid when the females were nine days old, and in one case oviposition was continued up to the thirty-eighth day, or for a period of twenty-nine days, the female dying the following day. Except during the four or five days preceding death, the number of eggs laid each day is fairly constant throughout the laying period in this observation; the average number being about five eggs per day for each female from the thirteenth to the thirty-third day.

Second Observation.

Three copulating pairs placed on a clean sheep on 17th March, 1913.

Date.	Age of Acari in days.	No. of Females laying.	No. of Eggs collected.	Remarks.
21/3/13.....	10	3	5	
22/3/13.....	11	3	14	
23/3/13.....	12	3	13	
24/3/13.....	13	3	10	
25/3/13.....	14	3	12	
26/3/13.....	15	3	15	
27/3/13.....	16	3	20	
28/3/13.....	17	3	15	
29/3/13.....	18	3	14	
30/3/13.....	19	3	12	
31/3/13.....	20	3	10	
1/4/13.....	21	3	13	
2/4/13.....	22	3	6	
3/4/13.....	23	3	3	1 female dead.
4/4/13.....	24	2	3	
5/4/13.....	25	2	0	
6/4/13.....	26	2	0	
7/4/13.....	27	2	1	1 female dead.
8/4/13.....	28	1	1	
9/4/13.....	29	1	0	
10/4/13.....	30	1	0	Female dead.
		Total...	167	

In this observation the average number of eggs per female acarus is 55.6.

The duration of the laying period is here seen to be much shorter than in the previous case, practically terminating when the females were twenty-three days old, although one acarus lived until the thirtieth day.

The two preceding observations were made during mid and late summer respectively. A third, carried out in early spring, is of

interest in connection with the supposed influence of moisture on the rate of multiplication of the parasites. This point will be referred to later, but the details of the observation will be recorded here.

During the three winter months preceding the commencement of the observation, practically no rain had fallen, and in spite of the fact that the infected sheep were in poor condition owing to scarcity of feed, it was noticed that the parasites became less numerous on them and the disease was ceasing to spread. Two or three attempts were made at this time to repeat the egg-laying observations, but in every case the acari removed from scabby sheep and placed under covers, laid only a few eggs and then died—it was also noticed that the females appeared smaller than normal. In the observation given below two copulating pairs of acari were placed under a cover on 15th September. On the 19th two eggs were found, and on the 22nd one egg. On the 24th one of the females was dead, but no further eggs were laid by the remaining acarus up to the 28th idem, when the long period of drought ended in a heavy downpour of rain—on this day two eggs were found. On the 29th idem it was still raining, and the patch was not examined, but on the next day twenty-four eggs were removed, and for the succeeding days the single female laid an average of seven eggs a day. Altogether this female laid ninety-three eggs, without counting the three found while the second female was alive. The commencement of active oviposition with the onset of the rains appears to be more than a coincidence in view of the small egg production in this and other observations made during the dry season, and the rapid laying exhibited by the acari when the atmosphere became moist. The sheep themselves were not actually exposed to the rain, but kept in covered pens.

The following are the full details of the observation:—

Third Observation.

Two copulating pairs of acari placed under cover on 15th September, 1913.

Date.	Age of Acari in days.	No. of Females laying.	No. of Eggs collected.	Remarks.
18/ 9/13.....	9	2	0	
19/ 9/13.....	10	2	2	
20/ 9/13.....	11	2	0	
21/ 9/13.....	12	2	0	
22/ 9/13.....	13	2	1	
23/ 9/13.....	14	2	0	
24/ 9/13.....	15	1	0	1 female dead.
25/ 9/13.....	16	1	0	
26/ 9/13.....	17	1	0	
27/ 9/13.....	18	1	0	
28/ 9/13.....	19	1	2	Raining heavily.
29/ 9/13.....	20	1	0	Eggs not removed; still raining.
30/ 9/13.....	21	1	24	Light rain.
1/10/13.....	22	1	8	
2/10/13.....	23	1	8	
3/10/13.....	24	1	6	Slight rain.
4/10/13.....	25	1	8	
5/10/13.....	26	1	7	

Date.	Age of Acari in days.	No. of Females laying.	No. of Eggs collected.	Remarks.
6/10/13.....	27	1	6	Raining.
7/10/13.....	28	1	5	Raining.
8/10/13.....	29	1	4	
9/10/13.....	30	1	2	Raining.
10/10/13.....	31	1	4	
11/10/13.....	32	1	2	Raining.
12/10/13.....	33	1	1	Little rain.
13/10/13.....	34	1	4	Little rain.
14/10/13.....	35	1	1	
15/10/13.....	36	1	1	
16/10/13.....	37	1	0	
17/10/13.....	38	1	0	
18/10/13.....	39	1	0	Female dead.
		Total...	96	

After the advent of rain, female acari were observed in several cases, and all laid eggs freely from the time they reached maturity.

In another observation three females lived for thirty-eight days, but the number of eggs laid was not determined; they were then accidentally allowed to escape from the cover, and so were lost.

It may be stated, therefore, that the number of eggs laid by the female psoropte varies within considerable limits, but under favourable conditions may exceed ninety eggs per female. Oviposition usually begins on the ninth day, and may continue with little interruption until the thirty-eighth day. A single female has been observed to lay twenty-four eggs in two days and seventy-six in ten days.

The life of the ovigerous female on the sheep is from thirty to forty days.

In his report for 1911, Sir Stewart Stockman, Chief Veterinary Surgeon, Board of Agriculture, draws attention to the fact that visible evidence of scab may not appear in a flock for several months after infection must have taken place, the acari apparently living on the sheep but producing no sign of the disease.

This question will be discussed later when other factors in the epizootiology of the disease are considered, but one point which may have a bearing on the matter will be dealt with here as it directly concerns the life-history of the parasite on the sheep.

It is probable that in many cases of infection of clean flocks the number of acari gaining access to any sheep is very limited, possibly only a single acarus. Should this be an ovigerous female, eggs will be laid, and in time the disease becomes established: but in the event of its being a pubescent female or a male, or a young form developing into one of these, no increase can occur until it is joined by an acarus of opposite sex.

It is of some interest, therefore, to determine how long a pubescent female or a male is able to survive on the sheep.

To obtain known unfertilized females it was necessary to isolate single nymphs until they moulted into adults, the two sexes thus being kept separate.

Considerable difficulty was experienced in keeping both forms confined under covers for any length of time. After the first day or two they became very restless, and owing to loosening of the covers some escaped, while others were crushed in attempting to do so.

The longest period for which a pubescent female was kept under observation alive on a sheep was seven weeks—the majority died or escaped three or four weeks after moulting from nymphæ.

Male acari were found to live for periods varying from twenty-two to thirty-four days after moulting from nymphs. The fact that they had fertilized female acari did not appear to affect the duration of their lives.

(b) FACTORS IN THE EPIZOOTIOLOGY OF SHEEP SCAB BEARING ON THE QUESTION OF ERADICATION.

Interval between Dippings.

Experience has shown that a single immersion of scabby sheep in an antipsoric fluid cannot be relied upon to effect a cure.

It is true that many cases of the disease will be cured by a single dipping in any of the several preparations in common use, but it is equally certain that this is not so with some dips, while probably there is no fluid that can safely be employed which is capable of curing all cases in one dipping. The necessity for second dipping is therefore generally admitted.

But the question of the interval to be allowed between the two immersions has given rise to much discussion and divergence of opinion.

The failure of the first dipping is usually attributed to the inability of the fluid to destroy the vitality of the acari eggs, and though this explanation has been questioned as being based on theoretical considerations, the observations on the effects of various fluids on the eggs described later show that the belief is well founded.

Experiment has shown that the majority of preparations, proprietary and otherwise, fail to prevent the eggs from hatching, hence the second dipping must be given before the parasites from the eggs which have escaped the first dipping, complete their development and themselves commence to lay.

In accordance with the generally accepted statement as to the length of time required for the acari to mature, the interval between dippings usually advised is fourteen to eighteen days, though in South Africa the time adopted is twelve to sixteen days.

The present observations have now shown that the complete life-cycle of the acari may only occupy nine days; owing, however, to the fact that sheep remain wet for some time after dipping, the period varying according to the length of their fleece, season, etc., an interval of ten days could probably be safely allowed. As will be shown later, this interval would ensure the hatching of all eggs capable of doing so before the second dipping.

The question may be asked, how it is that some dips will effect a cure in certain cases after one dipping, although they fail to destroy the vitality of the eggs?

The explanation appears to be that the eggs hatch, but owing to the persistence of certain ingredients of the dip on the skin, the larvae are poisoned shortly after hatching; this has been actually observed in several instances.

It has also been noted that this delayed lethal effect usually occurs in the case of dips containing an insoluble constituent in suspension which becomes deposited on the skin and in the wool of the sheep and remains after the animal has become dry.

Most commonly this insoluble agent is sulphur, and experiment has shown that a fine deposit of this material alone over eggs placed on the skin is sufficient to kill larvae shortly after hatching.

Solutions of arsenic that can be employed with safety, one per cent. solutions of various coal tar disinfectants, and carbolic dips and tobacco extracts have all been found ineffective by themselves in arresting the hatching and development of acari from eggs on the sheep.

The addition, therefore, of an ingredient like finely powdered sulphur which becomes deposited in the fleece, tends to increase the effectiveness of a dip, but its value is discounted somewhat by the difficulty of maintaining it in suspension in the fluid, and the fact that it does not reach parasites or their eggs which are protected by overlying crusts or matted wool.

While the failure of a single dipping to cure scab owing to the escape of the eggs has been demonstrated and the necessity indicated for a shortening of the interval between the two dippings on account of the rapid development of the parasites, observation has also shown that in certain cases after one immersion in a recognized dipping fluid, some adult parasites may survive, and unless destroyed by a further dipping continue the infection.

The escape of the parasites is usually due to the inability of the fluid to penetrate the hard crusts under which they lie, or as is frequently seen in the bend of the tail of the fat-tailed Africaander sheep, the protection of an excessive amount of fat is sufficient to prevent the watery solution from reaching the acari.

The increased softening and penetrating effect of slightly alkaline dips, such as the caustic soda and sulphur and lime and sulphur preparations, is of especial advantage in such cases, but even with these fluids the second dipping should not be omitted.

When a flock has been badly infected for a considerable time and a number of sheep show old standing crusts, even two dippings cannot be relied on to effect eradication; a few female acari may escape the first dipping and continue to lay eggs which the second immersion fails to destroy; or the protection of the crusts may allow adult parasites to survive even two immersions; in either case the infection persists, although it may not become evident for several months. Hence a third dipping ten or fourteen days after the second would greatly increase the chances of a complete cure; the softening effect of the two previous dippings on any hard crusts facilitating thorough saturation at a third immersion.

As previously mentioned, some allowance can be made when it is necessary to dip sheep having a considerable growth of wool as their fleeces hold more of the fluid, and they usually remain wet for a day or two after dipping. Special care is also necessary with such sheep when an arsenical dip is being employed, owing to the increased risk of poisoning, and before the interval between the first two dippings is reduced, or a third dipping adopted, it will be necessary to ascertain, more particularly in respect to proprietary arsenical

dips, whether these procedures can be safely carried out while following the directions given as to the dilution required under present circumstances.

(c) THE LENGTH OF TIME ACARI AND THEIR EGGS CAN RETAIN THEIR VITALITY APART FROM SHEEP.

The importance of possessing exact information on this point will be readily appreciated when it is recognized that on this depends the possibility of clean sheep becoming infected by any means other than direct contact with scabby sheep.

That the disease can be contracted from posts, kraals, sleeping-places, etc., to which infected sheep have previously had access is a well-established fact, but the very greatest divergence of opinion exists as to the period of time during which such infection can persist or after what interval it may safely be regarded as having ceased.

It has frequently been stated that sheep have contracted scab when placed in kraals occupied by infected sheep two or even three years previously, but which have been kept closed during the interval, and since this view is held by persons who have had wide practical experience of the disease in South Africa, their opinion cannot be lightly disregarded, although it must be admitted that the evidence adduced in support of prolonged infection of kraals is not convincing.

In regard to the vitality of the acari themselves apart from the sheep, numerous investigators record observations. Neumann states: "From the experiments of Hertwig and Gerlach, and particularly of Delafond it results, that the psoroptes of the sheep infecting crusts, portions of wool or pieces of fresh skin, kept at a medium temperature, can live in these for ten to twenty days; also that they soon perish if exposed either to a high or low temperature."

Stockman records having found living acari up to thirty-one days after removal from the sheep, but these only showed slight movement on warming. He was unable in three attempts to infect sheep with parasites that had only been removed from sheep for fourteen days, although large numbers were employed. On the other hand, Salmon and Stiles write that "Cases are on record where they (acari) have lived three, four, or even six weeks when separated from the sheep; if the atmosphere is dry, they will generally die in about fifteen days; but death is often only apparent, for the mites may sometimes be revived by warmth and moisture even after six or eight weeks." However, the authors themselves do not appear to have made these observations, nor do they give their authorities for the above statements.

The observations made by the writer agree very closely with those quoted by Neumann.

Acari of all ages were removed from sheep and kept in large jars at room temperature with plenty of crusts and wool; a moderate degree of moisture was maintained by suspending a piece of blotting-paper in the jar and damping it every two or three days with water; muslin tied over the top of the jar allowed of ventilation.

After three or four days the acari began to die, first the young forms, later the adults; by the tenth day the majority of the parasites appeared to be dead, those still active being mostly ovigerous females. In one observation in which several hundred acari were employed,

only a single female was feebly alive after being kept sixteen days away from the sheep, and next day this one was dead. In other observations acari have lived a day or two longer, and in one case a female acarus was alive twenty days after removal from its host; it was then placed on a sheep, but was found to be dead the next day.

When kept moist, but at body temperature (37° C.) in an incubator the acari are very active for two or three days, and eggs present in the crusts hatch. But a day or two later the larvae and most of the older forms die, and very few survive more than six or seven days.

In an ice-box at 0° C. all forms were dead in four days, while in a desiccator in an atmosphere free from water, at room temperature, they did not live longer than six days.

The majority of the parasites leave the crust soon after removal from the sheep and endeavour to escape from the jars.

In the incubator a good number of the ovigerous females lay eggs, and occasionally this has been observed to occur at air temperature during the summer, but in no case at a longer interval than two days after removal from the sheep.

The acari appear to live no longer upon shed wool and crusts in the open. The wool and adhering crusts containing great numbers of acari were pulled from a sheep immediately after its death and allowed to remain in an empty pen. Ten days later living acari could still be found in the crusts, but fifteen days after removal all appeared dead, nor did they show signs of life when placed in an incubator.

In view of the observations on the acari themselves most writers were forced to admit that prolonged infection apart from sheep could not be due to this form of the parasite, and they fell back on the assumption that it must be the eggs which retain their vitality for long periods.

The older writers make no mention of experiments regarding the vitality of eggs, nor of the prolonged persistence of infection in sheep folds.

Salmon and Stiles state: "Experience has shown that in some cases apparently healthy sheep have been infected in places where no sheep have been kept for four, eight, twelve, or even twenty-four months. The conditions underlying this infection are not thoroughly understood. Possibly some of the eggs have retained their vitality a long time and then hatched out; possibly the vitality of the fecundated female has also played a rôle; while it is not at all improbable that an entirely new infection has accidentally been introduced by birds or other animals." They regard this latter as the probable explanation, and conclude by recommending that sheds and fields in which scabby sheep have been kept should not be used for at least *four weeks* (better six or eight).

Davidson, writing of scab in South Africa, also records observations in which acari failed to survive longer than twenty-one days, and concludes that persistent kraal infection must be due to the ova of the mites retaining their vitality. Details are given of a kraal proving infective after being closed for three years, but in this case it was the sarcoptic scabies of goats that apparently persisted for that length of time and not sheep scab.

To determine the length of time the eggs of the sheep psoropt can remain apart from the sheep and still be capable of hatching, a large number of observations have been made by the writer.

The eggs were obtained by placing ovigerous female acari under covers on a sheep and removing the eggs laid each morning; they were then kept for varying periods under different conditions, and to test their vitality, they were again placed under a cover in contact with a sheep's skin and watched for several days. For each observation from ten to thirty eggs were employed.

Eggs kept at air temperature hatched after having been removed from the sheep for periods up to and including eight days. After nine days' removal, only one egg hatched out of twenty, and that one on the day after the eggs were placed on a sheep. Lots of eggs stored for ten, eleven, twelve, thirteen, and more days all failed to hatch. Several batches of eggs were also placed on moist sheep faeces and returned to a sheep after ten or more days, but in no case did any of the eggs hatch.

When kept in an ice-box for ten days, the eggs retained their vitality, ten out of thirteen eggs hatching on being placed on a sheep. In this case germination in the eggs must have been arrested by the cold, as they did not commence to hatch until they had been on the sheep three days, and several did not do so until the fifth day; when stored at air temperature the larvae usually appeared one or two days after being returned to a sheep. After twelve days in the ice-box thirty eggs all failed to hatch, as also did those kept for longer periods.

During the summer months eggs have occasionally hatched at air temperature, frequently over 90° F. in the shade, while on exposure to direct sunlight a number hatched in four days, although this is stated to destroy their vitality; the larvae, however, soon perished.

Shortly after the above observations were carried out Sir Stewart Stockman published, in his Annual Report for 1911, the results of tests of the vitality of acari eggs after storage for varying periods; he found that no eggs hatched which had been kept longer than eight days away from the sheep, the vitality of the eggs being tested by placing them in an incubator.

It is therefore no longer possible to regard the acari eggs as being capable of maintaining their vitality for long periods apart from the sheep, and thus accounting for persistence of infection in kraals, etc.

It has often been reported that the removal of manure from kraals formerly occupied by scabby sheep has been followed by the appearance of the disease amongst animals coming in contact with it, although affected sheep may not have been in the kraals for many months.

The explanation given is that the acari remain alive in the manure, and are liberated when this is taken out in winter or becomes disturbed after drying.

Apart from the failure of both acari and their eggs to retain their vitality for longer periods when stored in sheep manure than under other conditions, there are two or three points which make it difficult to believe that prolonged kraal infection is possible in the absence of living hosts.

Firstly, an examination of the mouth parts of the parasite shows that these are only adapted for piercing and imbibing liquid nutriment by suction; like ticks the acari have no means of dealing with

either liquid or solid material, except what they can obtain by puncturing the skin of an animal.

It is therefore not possible to imagine that they can feed on the sheep manure as has been suggested.

It is a recognized natural law that where an obligatory parasite is able to survive for long periods apart from its host this faculty is necessary for the development and maintenance of the species; thus, most varieties of ticks require to leave their host during moulting, and as the opportunities of reaching a new host may be infrequent, the newly moulted tick is able to live for several months without feeding. But such a provision is not necessary in the case of sheep scab. The complete life-cycle of the parasite occurs on the sheep and migration to a fresh host is only necessary when the number of parasites becomes excessive and the life of the host is threatened. But there is no need to provide for the prolonged existence of the acari during such migration, since owing to the gregarious habits of sheep opportunities of direct transmission from one animal to another are very numerous and quite adequate to ensure the maintenance of the parasites under natural conditions.

Lastly, the experience of the Australian Colonies and of numbers of farmers in South Africa has demonstrated that the disease can be stamped out by ensuring the complete destruction of the parasites on the sheep, irrespective of whether grazing has been changed or not; two or three dippings at short intervals being sufficient to prevent re-infection during the period the acari are known to survive away from the sheep. Could the infection of sleeping places, etc., persist for two years or more as is suggested, the eradication of scab would require the adoption of dipping measures similar to those necessary for the eradication of ticks, since re-infection would be frequent, but this has not been shown to be the case.

It is significant that most of the alleged examples of prolonged infection of kraals occur at a season of the year when sheep become poor in condition, and in flocks either previously treated for scab or in which all opportunity of infection apart from the kraal has not been rigidly excluded for some time past.

In those cases in which infection by other sheep has been impossible for a considerable period the occurrence of the disease can be explained by assuming that the acari have lived on the sheep since the last known opportunity of infection, but without increasing in numbers or producing visible symptoms of scab; when the conditions become favourable to the parasites rapid multiplication takes place and their presence is manifested. As will be shown later such prolonged latent infection of sheep is known to occur, and in the writer's opinion accounts for the appearance of the disease in many supposed clean flocks without regarding the old kraals as the source of the infection.

In view of the importance of obtaining accurate information on the subject, it was decided early in the present investigation to carry out tests under as natural conditions as possible; accordingly two stone kraals were built, and pens and enclosures made which could be grossly infected by scabby sheep, and after being closed for varying periods tested as to their infectivity by having clean sheep placed in them.

The details of these observations are given below in tabular form :

Expt. No.	Particulars of Infection.	Interval Between Removal of Infected Sheep from Kraal and Contact with Clean Sheep.	Length of Time Clean Sheep Remained in Contact.	Result.
1	37 Scabby sheep herded in kraal for 44 days	21 days.....	76 days.....	Sheep clean.
2	30 Scabby sheep herded in kraal for 48 days	60 days.....	16 days.....	Sheep clean.
3	Do.	90 days.....	7 days.....	Sheep clean.
4	37 Scabby sheep herded in kraal for 35 days	10 days.....	60 days.....	Sheep clean.
5	Infected sheep running in a kraal for several months	24 days.....	48 days.....	Sheep clean.
6	Wool crusts and acari spread in box	Box kept free of sheep for 20 days	4 months.....	Sheep clean.
7	Acari and pieces of fleece scattered in box	Box kept free of sheep for 15 days	4 months.....	Sheep clean.
8	Manure collected from a kraal in which infected sheep had been running up to 16 days previously	Clean sheep immediately put into (a) clean kraal with manure, (b) above sheep removed after 5 days and replaced by second lot of clean sheep	5 days..... Six months....	Sheep infected. Sheep clean.
9	2 Scabby sheep running in a pen for 7 weeks. At the time of removal pieces of fleece and acari were spread on floor of pen	8 days.....	4 months.....	Sheep clean.
10	Do.	12 days.....	4 months.....	Sheep clean.

(d) THE POSSIBILITY OF THE *Psoroptes communis*, VAR. *ovis*, MAINTAINING ITS EXISTENCE ON ANIMALS OTHER THAN SHEEP OR OF THE PSOROPTES OF SUCH ANIMALS PRODUCING SCAB IN SHEEP.

It has been suggested that the alleged persistence of sheep scab in the prolonged absence of sheep may be accounted for by the acari being able to exist on other animals, such as rodents, and subsequently regain access to sheep.

For this reason the question of the adaptability of the psoroptes to different species of host is worthy of investigation, but in South Africa it derives additional importance on account of the wide-spread occurrence of *Psoroptic otacariasis* (ear scab) amongst goats.

It is very commonly believed that infection can spread from one species of animal to the other, and the frequent presence of the disease in both sheep and goats grazing together appears to support that view; there are, however, many instances of mixed flocks in which one species is badly infected while the other remains quite clean.

The psoroptes of sheep, goats, and rabbits were employed in the present observations.

Psoroptic otacariasis of goats has received scant notice in works on parasitology. Neumann quotes the single observation of Pesas and Morot, by whom the acarus was found in the ears of two goats from the Western Pyrenees. Law gives the same reference.

In addition to the above, Neveu-Lemaire mentions the observation of auricular psoroptes in goats in the Congo by Mense, who named the parasite *P. congolensis*. Geddoelst, however, has shown this to be the same acarus as that found by Pesas. Neveu-Lemaire regards the disease as frequently fatal and the prognosis serious unless treated early.

In South Africa the occurrence of psoroptes in the ears of goats was first reported in 1911, since when it has been found to be very widely distributed in the subcontinent; it is probable, therefore, that it has existed unrecognized for a long time.

Up to the present only the common Kaffir goats have been found to be infected.

The parasites confine themselves to the interior of the ear, usually well down in the meatus and the fossae at the base of the conchal cartilage; they have never been found on the skin outside the ear, and attempts to infect goats on the body have failed.

They do not appear to set up the intense irritation evident in sheep scab, but infected animals frequently shake their heads and many scratch the back of their ears with the hind feet.

There is an abundant desquamation of epithelium which, with the cerumen, forms hard masses, sometimes filling up the interior of the ear, as in the case of rabbit otacariasis; in this material the parasites are often present in large numbers.

No instances of suppuration or of the other sequelae that occur so frequently in otacariasis of the rabbit, dog, and cat have been met with.

The parasites differ but slightly from those of the same species in sheep. The adult forms are usually of a brownish colour, probably from their being able to pierce the thin lining membrane of the ear and obtain blood. Both male and females are slightly larger than the ovine psoropt; measurements made agreed very closely with those given by Neumann, viz., the male 470 microns to 640 microns long by 270 microns to 400 microns broad, and the ovigerous female 680 microns to 850 microns by 390 microns to 550 microns.

The destruction of the acari in the ear is easily effected, the best remedies being those containing a disinfectant mixed with glycerine or oil. Probably the simplest is a mixture of petroleum and cocoanut oil in the proportion of 1 to 2; one dressing with this is sufficient to destroy both the acari and their eggs.

Psoroptes communis, var. caprae, on Sheep.

When transferred to the skin of sheep, the goat psoroptes quickly begin feeding, pustules and crusts being formed as with the sheep acarus. The females lay eggs and these hatch after the same interval as that required by the eggs of the sheep psoropt, namely, two days. Similarly, eggs removed from a goat's ear will hatch when placed on a sheep.

The larvae quickly develop and in two days moult into nymphae.

At this stage, however, they usually begin to die, but in some instances further development has taken place and the nymphae have moulted into adults.

In two observations only the young acari have mated, in one case five days after hatching; but in both instances the parasites died without eggs being laid.

Ovigerous females and males transferred from a goat's ear, have remained alive on sheep up to ten days, but usually they die in from four to six days.

The young goat acari that have hatched on sheep are indistinguishable from sheep psoroptes and do not become dark in colour like those in the ears of goats.

In every case sheep that have had goat acari placed on them have been kept under observation for several months after the acari have died out and the covers been removed, and in two cases the covers were removed whilst there were large numbers of larvae and nymphae still alive, but in all the observations the sheep have failed to develop any evidence of scab.

The following details of sheep infected with *Psoroptes communis*, var. *caprae*, may be given:—

Sheep 136.

19th August, 1913.—Five ovigerous female acari and three pairs in copula removed from the ear of goat No. 206, placed under cover No. 80 on back.

21st August, 1913.—Six females and male observed.

22nd August, 1913.—Six females and male observed, also several eggs.

24th August, 1913.—Numerous larvae present.

25th August, 1913.—Only three live females observed and a few larvae and nymphae.

27th August, 1913.—No living acari observed; several dead. Patch examined at intervals for several days, cover then removed; no acari.

27th August, 1913.—Four ovigerous female acari from goat No. 206 placed under cover No. 81 on back.

29th August, 1913.—Numerous eggs laid.

30th August, 1913.—The four females removed and placed under cover No. 85 on the same sheep, numerous eggs remaining.

1st September, 1913.—Several larvae and few eggs.

3rd September, 1913.—Larvae growing.

5th September, 1913.—A few nymphae alive; several larvae and nymphae dead.

11th September, 1913.—Several dead nymphae; no living acari observed; cover removed.

No development of infection.

30th August, 1913.—Cover No. 85; four females from cover No. 81.

31st August, 1913.—Three females alive; 1 dead; no eggs.

1st September, 1913.—Three females dead.

No development of infection.

1st September, 1913.—Four ovigerous female acari from ear of goat No. 206 placed under cover No. 86 on back.

3rd September, 1913.—Four females alive; several eggs laid.

4th September, 1913.—Four females alive; eggs numerous.

- 5th September, 1913.—Four females alive; larvae hatched.
 6th September, 1913.—Four females alive; numerous larvae and eggs.
 8th September, 1913.—Four females alive; nymphs and larvae present.
 9th September, 1913.—Two females dead; several nymphae present.
 11th September, 1913.—One pair in copulo and several nymphae growing.
 The cover removed and acari allowed to escape.

This sheep, which had been infected four times with goat psoroptes, was examined at intervals for three months, but no acari were found on it after the removal of the last cover on 11th September, 1913.

Sheep 134.

- 9th September, 1913.—Ten nymphae from sheep 136, cover No. 86 (goat psoroptes) placed on back under cover No. 93.
 10th September, 1913.—Ten nymphae alive and growing.
 12th September, 1913.—Two pairs in copula and five pubescent females.
 14th September, 1913.—Cover torn off, sheep evidently had been biting at the spot; no acari could be found.

This sheep was also kept under observation for three months without showing any sign of infection.

Sheep 141.

- 24th September, 1913.—Six ovigerous female acari from ear of goat 208 placed on back under cover No. 106.
 25th September, 1913.—Six females alive; a few eggs laid.
 26th September, 1913.—Six females alive; a few eggs laid.
 27th September, 1913.—Five females alive, 1 dead; no larvae observed.
 30th September, 1913.—Four females dead, one missing; no living acari observed; cover removed.

No infection occurred in this sheep up to three months from the time living acari were last observed.

In addition to the actual placing of goat acari on sheep, two clean sheep were kept closely penned with two infected goats for over three months without contracting scab.

Acari from the ears of goats were also placed in a sheep's ears on several occasions, but in every instance they quickly died or disappeared.

Conclusion.—The *Psoroptes communis*, var. *caprae*, is able to live and reproduce itself on sheep, but all attempts to set up scab in sheep with the goat psoropt have failed; the original infecting parasites die out in less than ten days, while the second generation usually dies on reaching the nymphal stage, and in no case has a third generation been observed.

Psoroptes communis, var. ovis, to Goats.

Repeated attempts have been made to infect goats by placing all forms of acari taken from scabby sheep into their ears, but in no instance have the parasites been observed to live for more than two days. To prevent the goats from dislodging the parasites by shaking their heads, the ears were lightly plugged with wool after the parasites had been placed in the external meatus, and the edges of the concha loosely sewn together.

Goats that had been frequently infected with sheep acari and others kept in close contact with scabby sheep were examined at intervals for several months always with negative results.

Sheep acari placed on the bodies of goats quickly died or disappeared.

Conclusion.—The *Psoroptes communis, var. ovis*, is incapable of setting up otacariasis in goats or of maintaining its existence on this animal.

Psoroptes communis, var. cuniculi, to Sheep.

There are several references to the occurrence of psoroptic scabies in horses as the result of infection from rabbits.

Neumann quotes instances reported by Mathieu, Cagny, and Cadeac, and while admitting the absence of precision in their details, thinks them sufficient to prove the identity of the psoropt of the rabbit with that of the horse.

From the particulars given, however, it would appear that the disease was of a temporary character, or that the horses were subject to repeated infection from the rabbits. The observations with goat psoroptes on sheep and those recorded below with rabbit psoroptes show that the production of symptoms and the brief existence of parasites from one species of animal on another are not sufficient evidence that the disease in the two hosts is caused by identical varieties of psoroptes.

When placed on sheep the rabbit psoroptes attack the skin and cause irritation and the formation of crusts in the same way that the goat ear psoroptes do; eggs are laid and hatch in two days. The young acari develop quickly, mating having been observed six days from the time of hatching.

But as with the goat psoropt, development does not proceed beyond the adult stage of the second generation. In one observation only was a single larva found which may have hatched from an egg laid by the second generation (*vide infra*).

Adult acari have lived for seventeen days on sheep, and during this time laid numbers of eggs, but, as stated, the second generation of acari have died out without reproducing themselves.

In several observations eggs only were placed on sheep. With the exception of the doubtful instance referred to above the acari from those eggs all died before they reached the laying stage.

All the sheep used in the observations were examined at intervals for several months after infection, but in no instance did scab develop.

Similarly a sheep kept in a small run with several infected rabbits for over two months failed to show symptoms of scab.

Details of a few of the observations are given below:—

Sheep 163.

- 19th May, 1913.—A large number of acari (all stages) from ear of rabbit placed under cover on back.
 20th May, 1913, to 31st May, 1913.—Numerous eggs laid and young forms developing; majority of adults alive and active.
 2nd June, 1913.—Several adults dead; numerous young forms active; some pairs.
 4th June, 1913.—Many acari dead; several young forms alive.
 9th June, 1913.—No living acari observed; cover removed. No subsequent development.
 25th June, 1913.—Thirty acari eggs from ear of rabbit placed under cover.
 27th June, 1913.—Twenty larvae hatched.
 29th June, 1913.—Larvae developing.
 1st July 1913.—Nymphae developing.
 3rd July, 1913.—Nymphae and young adults present; cover removed to allow acari to escape.
 Sheep examined at intervals for several months; no development of scab.

Sheep 316.

- 5th July, 1913.—Six pairs of acari from ear of rabbit placed under cover.
 7th July, 1913.—Four pairs still united; a few eggs laid.
 8th July, 1913.—Numerous eggs laid.
 9th July, 1913.—A few larvae hatched.
 10th July, 1913.—Two females dead; several larvae developing.
 14th July, 1913.—Only two females alive; males and young forms dead; eggs still being laid.
 19th July, 1913.—No living acari observed; cover removed; no subsequent development.
 23rd July, 1913.—Forty-two acari eggs from ear of rabbit placed under cover.
 26th July, 1913.—Numerous larvae hatched.
 28th July, 1913.—Larvae developing; some nymphae.
 21st July, 1913.—Three pairs mating; numerous nymphae.
 2nd August, 1913.—Several adults; no eggs.
 5th August, 1913.—All adult acari dead; one larvae alive only; no eggs observed.
 6th August, 1913.—No live acari observed; cover removed; no development of scab occurred.

Conclusion.—The *Psoroptes communis*, var. *cuniculi*, is able to live on sheep for periods up to seventeen days, and during that time lays eggs and gives rise to symptoms and lesions similar to those caused by the ovine psoropt. The second generation of acari, however, die before reproducing themselves, and in consequence continuous acariasis of sheep cannot result from infection with the rabbit psoropt.

Psoroptes communis, var. ovis, to Rabbits.

As in the case of goats, all attempts to produce otacariasis in rabbits by infecting them with psoroptes from sheep failed.

Owing to the prevalence of the disease in rabbits it was necessary to isolate the animals some time before they were to be used and examine their ears at intervals to ensure their freedom from natural infection.

Acari of all ages and eggs were placed in the ears of rabbits, a little wool being inserted and kept in position by a few stitches to prevent the acari from being shaken out. In every case the acari died in two or three days, and no larvae were ever observed to have hatched in the ears.

Attempts were also made to infect wild rats with the sheep psoropt by placing scabby wool and acari in their cages, but in every case the result was negative.

Psoroptic scabies have never been reported as occurring in pigs; many South African farmers, however, believe that sheep can become infected with common scab from mangy pigs. It is quite likely that sheep may contract face scab (sarcoptic scabies) from pigs, since the different varieties of sarcoptic scabies are much more accommodating in the matter of host than are psoroptes (see footnote).

Attempts were, however, made to infect a pig with psoroptic mange by keeping it closely penned with scabby sheep and placing large numbers of acari on it, but the result was entirely negative.

Conclusion.—There is no evidence to show that the sheep psoropt is able to maintain its existence on any animal other than sheep and so continue sheep scab infection in the absence of its particular host. All attempts to infect other species of animal with sheep psoroptes have failed.

(c) VARIATIONS IN THE RAPIDITY OF MULTIPLICATION OF THE ACARI.

In Great Britain one of the most curious features of sheep scab is the almost complete disappearance of the disease during the summer months.

Sir Stewart Stockman, in his annual report for 1909, discussed this phenomenon, and gives several suggestions that have been offered in explanation, but none regarded as wholly satisfactory. Observations are quoted showing that without treatment of any kind, certain infected sheep became spontaneously cured in the spring months, and remained free from the disease during the summer following.

Further observations are recorded in his report for 1911, and from these it is concluded that there is "nothing in connection with the habits of the parasites or the general condition of sheep to account satisfactorily for the apparent disappearance of scab in summer in the field."

Shearing was found to distinctly retard the progress of scab, and in some cases apparent recovery followed.

The disappearance of the disease in summer was accounted for mainly by shearing and dipping, and the increased outbreaks in the early winter are attributed to the latter operation being ineffective.

The author has produced sarcoptic scab in sheep with goat sarcopt and continued the infection through sheep and back again to goats; pigs and cattle have also been infected with sarcoptic mange by means of the goats acarus.

except as checking the disease. The autumn sales lead to the distribution of such apparently clean sheep to previously uninfected farms.

In South Africa marked variations in the prevalence of sheep scab are also observable, but the conditions under which these occur are such that they cannot be explained in the same way as in Great Britain since dipping and—in the case of Africander sheep—shearing are not carried out as general practices at definite seasons of the year.

The following is an instance:—

During the past two years a flock of scabby sheep has been maintained at the Laboratory to supply material for the experiments, and subjects for testing the efficiency of dipping fluids. As grossly infected animals were removed, others were drafted in to contract the disease, but a good proportion harbouring acari were always left to carry on the infection.

It was found, however, that in the early spring—August and September—each year the number of acari on the sheep began to diminish, so that on many which had previously been badly infected, parasites could only be found after considerable search, while in some cases they could not be found at all. This decrease in the activity of the parasites occurred in all the sheep, and was not confined to those in which the disease had spread over the greater part of the animal, thus rendering the skin distasteful to the acari, but was equally noticeable in those only recently infected.

As illustrating the rapidity with which the parasites are able to multiply, the computation made by Gerlach is often quoted. Assuming that each female lays fifteen eggs and that development occupies fifteen days, he estimated that one and a half million acari would arise from a single pair in three months.

When, however, we find that, in South Africa at all events, a female acarus can lay over ninety eggs and the life-cycle may be completed in eleven days, it is evident that even Gerlach's figure is far short of the actual possibility, given conditions favourable to the parasites.

Something approaching this extraordinary rate of multiplication has been observed in two or three natural cases, when in the course of a week acari in countless numbers have invaded the entire surface of a sheep's body and rapidly caused its death.

But such cases are uncommon and occur only when sheep are in very low condition.

In the great majority of sheep naturally infected the multiplication of the parasites is far short of that observed under experimental conditions, and very often the number present does not appear to increase at all for quite long periods. Under certain circumstances the infection may become less, as instanced above, or to all appearances cease entirely.

This fact is well known to sheep farmers in very dry areas, such as occur in the north-west of the Cape Province. Here at certain seasons of the year flocks that have been badly infected may become apparently clean and remain so for a month or two without treatment of any kind having been carried out. But when on account of drought the sheep lose condition scab again makes its appearance, although no opportunity of reinfection may have occurred in the meantime.

The influence of the bodily condition of the host on the rate of multiplication of the parasites is well recognized, but no very clear

explanation has been offered as to the exact way in which the development of the acari is checked.

The presence of grease in the fleece is believed by several South African authorities on sheep to be the chief determining factor. It is stated that when giving a demonstration on sheep scab the late Dr. Hutcheon was once asked to attempt to infect some very fat pet lambs, and although a large number of acari were placed on each of these animals no development of the disease occurred—this result was attributed to the large amount of grease in the lambs fleeces.

Most writers refer to condition, age, breed, and length of fleece of sheep and the season of the year as affecting the course of the disease, but they do not describe how these factors produce their effect.

In carrying out the observations on the life-history of the acari it was frequently noticed that development occurred just as rapidly on the washed and shaven patches on fat sheep as on those in poor condition, but when the covers were removed and the parasites allowed to spread to the surrounding skin, multiplication was much more rapid in the case of the thin sheep than on those with excess of yolk in their fleeces, while in a few of the latter no development occurred at all.

In the same way some attempts to infect sheep with very greasy fleeces by placing numbers of acari on them have failed. The following instances may be quoted:—

Sheep 4420 (Merino), with twelve months' growth of wool in good condition, fleece very greasy.

29th August, 1914.—Ten ovigerous female acari placed under two covers.

31st August, 1912.—Numerous eggs and a few larvae present.

7th September, 1912.—Acari of all ages present.

8th September, 1912.—Covers removed, allowing acari to escape.

9th September, 1912, to 30th October, 1912.—Sheep examined at intervals without any live acari being discovered.

31st October, 1912.—Eight ovigerous female acari placed on skin near rump.

1st November, 1912.—One acarus only found; sheep nibbling.

3rd November, 1912.—No acari found; sheep nibbling.

4th November, 1912, to 22nd December, 1912.—Examined at intervals; no sign of infection.

23rd December, 1912.—Sheep shorn and thoroughly examined without a sign of acari being found.

Sheep 136 (Merino-cross), with twelve months' growth of wool; fleece very greasy.

5th September, 1912.—Seven ovigerous female acari placed near rump.

6th September, 1912, to 30th October, 1912.—Examined at intervals without any acari being found.

30th October, 1912.—Sheep shorn and carefully examined, without result.

When a small number, say five to ten, ovigerous female acari have been placed on sheep in only moderate condition and with little yolk

in their fleeces the development of the disease has always followed—usually symptoms were exhibited ten or twelve days after infection.

In order to observe more closely the effect upon the acari of the presence of wool fat, two patches were prepared upon a sheep; one was washed and shaved in the usual way, but on the other the wool was only cut short, and in addition the patch was smeared with "yolk" obtained from the wool of another sheep. As each generation of acari began to develop on the ungreated patch, a number of the young forms were removed to a fresh area so that the number of generations could be determined.

The following tabular statement gives the details of the observation:—

Development of Acari on Greased and Ungreated Patches.

Date.	Greased.	Ungreated.	Remarks.
2/10/12	6 ovigerous female acari placed on patch	6 ovigerous female acari placed on patch	2nd generation on ungreated patch.
3/10/12	A few eggs present	A few eggs present	
4/10/12	Numerous eggs present	Numerous eggs present	
6/10/12	Several larvae and eggs....	Several larvae and eggs.....	
7/10/12	Larvae and eggs present...	Larvae and eggs present.....	
9/10/12	Larvae not so numerous, eggs still present	Numerous nymphae, larvae, and eggs	
10/10/12	A few nymphae and larvae and small number of eggs	6 original females removed, nymphae growing	3rd generation on ungreated patch.
13/10/12	One female dead, 2 missing, only 14 nymphae and larvae found; original females removed	2 copulating pairs and numerous nymphae, larvae, and eggs; 14 acari, including the 2 young pairs, removed to a fresh patch	
15/10/12	12 acari found still nymphae	3 pairs and 8 females, several eggs laid	
16/10/12	9 acari found, nymphae or pubescent females.	1 female dead, 3 males and 10 females observed. numerous eggs	
17/10/12	9 nymphs of pubescent females	9 females and 3 males observed; numerous larvae and eggs	4th generation on ungreated patch.
20/10/12	5 females and 1 male observed	20 nymphae and larvae removed to a fresh patch	
22/10/12	1 pair in copulo and 4 females	Nymphs developing.....	
24/10/12	5 females observed, no eggs..	Females observed.....	
26/10/12	1 adult female only observed, no eggs	2 copulating pairs and several oviporus observed	
27/10/12	1 adult female only observed, no eggs	Several eggs found.....	4th generation on ungreated patch.
29/10/12	No live acari found.....	Numerous larvae present.....	
31/10/12	Examined every 2 or 3 days without any acari being found	Numerous larvae present. Acari developed as before, each cycle from egg to egg occupying 9 to 10 days	
18/12/12			

It will be seen that in both cases eggs were laid on 3rd October, 1912, and larvae were present on 6th October, 1912. On the ungreated patch development occurred as usual, eggs being laid nine days after the appearance of the larvae, and this cycle was continued for several generations.

On the greased patch, however, the majority of the larvae died two or three days after hatching, while those that survived developed very slowly; thus it was not until 20th October, 1912, fourteen days after hatching, that adult male and female acari were observed, and though fertilization took place no eggs were laid and living acari were not observed after 27th October, 1912. By this time the fourth generation of acari were developing on the ungreased area.

This observation has been repeated several times. When the skin was greased very slightly the acari developed as rapidly as on the ungreased area. In these cases the growing wool lifted, the first crusts formed, and these carried the fat with them, so that the acari were able to live on the dry skin beneath. However, when sufficient grease was present to soak down under the crusts development was very slow, and in every case the acari gradually died out, usually in about twenty days.

Under natural conditions the fat, being excreted by the skin, is likely to have a relatively greater effect on the acari than when applied on the surface as in the above observations.

The point, however, to which it is desired to draw particular attention is not the lethal effect of wool fat on the acari, but that the presence of this material markedly retards the life-cycle of the parasites. In the experiments it was not possible to regulate the quantity of fat on the skin so as to check development and yet avoid actually killing the acari, but it seems reasonable to assume that under natural conditions such a moderate degree of fat excretion may occur. Should this be so, it would account for the observed variations in the rapidity of multiplication of the acari on infected sheep, and explain how the parasites may continue to live on sheep without giving rise to visible signs of scab for considerable periods.

The reappearance of scab in sheep several months after apparent recovery, either as the result of dipping or without treatment, has been frequently observed in South Africa.

Stockman gives numerous instances of the same kind in Great Britain.

In most cases no contact with other scabby sheep could have occurred. It has been usual in South Africa to explain such recurrences as due to reinfection from kraals and sleeping places. As has been recorded earlier in this report no experimental evidence can be obtained in support of the theory of prolonged infection apart from sheep. In view of the observed persistence of acari on sheep without the production of visible lesions, either as the result of dip remaining in the fleece or (as is suggested by the present experiments) the excretion of an excess of fat by the skin, it appears to be more reasonable to explain recurrences of the disease in flocks that have not been exposed to outside infection since their apparent recovery, as examples of latent infection of the sheep themselves rather than to assume that the parasites have lived apart from their host for much longer periods than actual observation warrants. No doubt in many of such cases the infection has actually been introduced from an outside source without the knowledge of the owner.

Other factors besides the presence of fat in the fleece appear to influence the rate of multiplication of the acari.

In detailing the observations on the egg production of female acari mention was made of the apparent effect of moisture, the onset of rain being followed by a great increase in the number of eggs laid.

Sheep farmers in the Cape Province many years ago recognized that the first rains were frequently followed by the appearance of scab in their flocks, and not unnaturally they attributed the disease to the moisture and young vegetation, its parasitic nature not being recognized.

Observations on acari kept in a desiccator have shown that they rapidly succumb when all moisture is removed from the air. An attempt was therefore made to keep them on a sheep, but in a dry atmosphere. With this object a cover was made, having a second chamber separated by a muslin division from that over the parasites. This was filled with calcium chloride, which was charged every day. In this way the skin and air over the acari were kept fairly dry from moisture. The parasites, however, developed as rapidly as under an ordinary cover, though owing to the difficulty of maintaining the double chamber in position it was not possible to continue the observation for longer than one generation—prolonged absence of moisture might have caused a decrease in the rate of multiplication of subsequent generations.

While it has not been possible to actually demonstrate any effect from the presence or absence of moisture in the air, there is evidence that under natural conditions the disease lessens in dry weather. This may, however, be mainly the result of the accumulation of fat in the fleece, and in the same way the increased activity of the parasites following rain may be accounted for by the resulting washing of the fat from the fleece that then takes place. The increase of scab following prolonged drought is explained by the loss of condition of the sheep, in consequence of which the excretion of fat from the skin diminishes, thus allowing the parasites to multiply rapidly.

CONCLUSION.

One or two practical points suggested by these observations may be briefly mentioned.

They have shown that the all-important factor in the eradication of sheep scab is the destruction of every acarid on the sheep themselves, and to ensure this more efficient dipping is necessary than has usually been employed in the past. The necessity of reducing the interval between the two dippings from the fourteenth or sixteenth days—commonly considered adequate—to ten days has already been pointed out, and the advisability of giving a third immersion advocated. The expense of this additional dipping is slight compared to that occasioned by a recurrence of the disease, possibly some months later, owing to the survival of a few acari on one or two sheep that perhaps passed too rapidly through the tank or were not completely immersed.

In addition, a third dipping would greatly lessen the risk of reinfection from kraals or sleeping places. Acari are known to be unable to live for more than four weeks apart from sheep (in the experiments all were dead in twenty-one days), and eggs will not hatch after ten days, so that by the time the third dipping be given—say twenty to twenty-four days after the first—acari in the kraals would be either dead or too feeble to infect recently dipped sheep.

It would, of course, be unwise to neglect to thoroughly disinfect all places in which the scabby sheep had been confined, but absolute cleansing of the sheep is the essential point in the eradication of the disease.

The necessity of treating every sheep in the flock, and not only those visibly affected, is obvious.

When possible sheep should be dipped in early summer, shortly after shearing. They are then best able to stand the immersions and no damage is done to the wool, while scab and other parasites are most easily destroyed.

If the flock is only slightly infected or recently exposed to infection, dipping should not be delayed during the summer because of the absence of signs of active scab. In the winter, when the sheep carry more wool and are probably in poorer condition, the disease will increase rapidly, and dipping will then have to be carried out under the most unfavourable conditions both as regards the health of the sheep, damage to the wool, and the eradication of the disease.

The choice of a dip is a most important matter. A large number of dipping fluids—proprietary and otherwise—have been tested: the result of these tests will be reported at a later date.*

One item of considerable interest may, however, be mentioned here. It has long been recognized that dipping fluids do not destroy the vitality of acari eggs, hence the necessity of giving a second dipping. In the observation all the fluids tested failed to prevent the hatching of eggs, with the exception of the lime and sulphur dip. This fluid, prepared according to the instructions of the Division of Sheep, and also when a weaker solution containing only 20 lb. of sulphur to 100 gallons of water was employed, destroyed the vitality of all eggs in a large number of tests both on sheep and *in vitro*. The value of this property of the lime and sulphur dip cannot well be over-estimated, and it may partly account for the success attained in the eradication of scab from Australia; this dip was largely employed in effecting that happy result.

* *Vide* report of the Entomologist of the Division upon the dipping trials carried out with the different proprietary and home-made sheep dips in South Africa.

Experiments and Observations
carried out with
***Psoroptes communis* at Onderstepoort.**

BY

G. A. H. BEDFORD,

Entomologist, Division of Veterinary Research.

Experiments and Observations carried out with *Psoroptes communis* at Onderstepoort.

By G. A. H. BEDFORD, Entomologist, Division of Veterinary Research.

P. communis, var. *ovis*.

P. communis, var. *ovis*, is by far the worst sheep pest the farmer has to deal with in South Africa.

The only other species with which this acarus can be confused is *Chorioptes symbiotes*, the different varieties of which are found on the horse, ox, sheep, and goat, but I know of no instance of this species having been found in South Africa, except from a report by the late Hutcheon (¹), in which he states that "The Boer goat does frequently become infected with the genus *Symbiotes* from the Angora goat."

The genus *Chorioptes* can at once be distinguished from *Psoroptes* by the pedicles of the legs, which carry the suckers at their apices, being very short (consisting of one joint), whereas in *Psoroptes* they are long and tri-jointed; also the suckers are larger in *Chorioptes* than what they are in *Psoroptes*. These pedicles are present on the first two pairs of legs, also on the third pair in the male and fourth pair in the nymph and ovigerous female.

DESCRIPTION OF THE DIFFERENT STAGES.

The ova is creamy white in colour; oval in shape with a smooth and shiny surface; length 0.28 mm.; width 0.11 mm. (fig. 1).

The larva has three pairs of legs, the third pair terminates in two long bristles; length 0.25 to 0.29 mm.; width 0.15 to 0.23 mm. (fig. 2).

The nymph has four pairs of legs; at the apex of the fourth pair there is a pedicle with a sucker and a bristle; length 0.41 to 0.46 mm.; width 0.3 to 0.34 mm. (fig. 3).

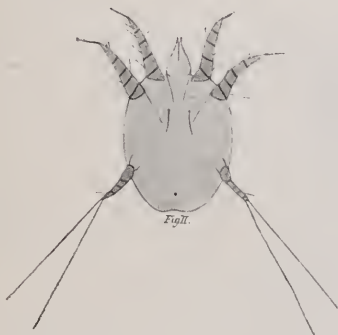


Fig. 2—*Psoroptes communis*, var. *ovis* :
larva.



Fig. 3—*Psoroptes communis*, var. *ovis* :
nymph.

The pubescent female resembles the nymph, but can be distinguished by the following:—The fourth pair of legs are terminated in two long bristles, pedicles absent; the presence of a large vulvo-anal slit and two copulatory tubercles situated at the apex of the abdomen beneath the dorsal surface, their function being to assist

(¹) Hutcheon, D. Scab; its nature, cause, symptoms, and treatment. Capetown. 1895.

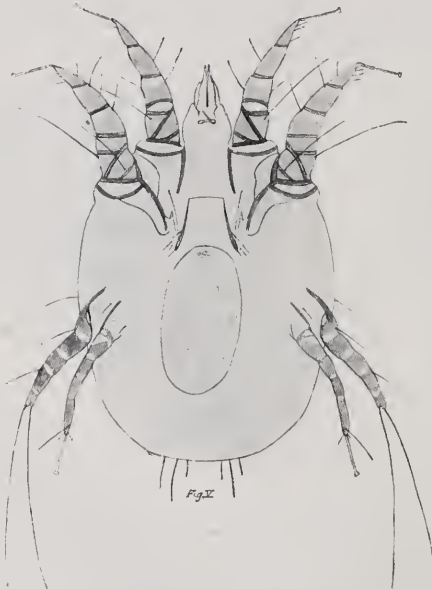
in copulation by being received into the copulatory suckers of the male; length 0.48 to 0.53 mm. (fig. 4).



G. A. H. Bedford, del.

Fig. 4—*Psoroptes communis*, var. *oris*: pubescent female.

The ovigerous female.—The fourth pair of legs each have one sucker and one long bristle at their apices; on the ventral surface there is a large sub-thoracic vulva situated between the second pair of legs which is absent in the pubescent female; length 0.61 to 0.65 mm.; width 0.38 to 0.41 mm. (fig. 5).



G. A. H. Bedford, del.

Fig. 5—*P. communis*, var. *oris*: ovigerous female.

The male.—The third pair of legs are very long and terminated by a sucker and a long bristle; the fourth pair of legs are very short with two short bristles on the last joint; in addition there are copulatory suckers and triangular abdominal lobes which project beyond the apex, each is furnished with five bristles, the three median ones being very much longer than the other two; length 0.45 mm.; width 0.35 mm. (fig. 6).

In addition to the above characters the following are common to the different stages:—The body is oval in shape, surface very finely acidulated; on the dorsal surface there are ten bristles placed symmetrically, bristles are also present on the ventral surface, but less numerous; the rostrum is elongated and destitute of cheeks.

Life-history.

The life-history was worked out at Onderstepoort at all seasons of the year with both short and long woolled sheep, and the life-cycle was found to be exactly the same throughout the year, and also to coincide with Shilston's observations conducted at Pietermaritzburg, although the climatic conditions vary somewhat, as will be seen by the meteorological records here recorded for the year 1913:—

1913. MONTH.	RAINFALL.		TEMPERATURES.					
	Pre- toria.	Maritz- burg.	Pretoria.			Maritzburg.		
			Mean Maxi- mum.	Mean Mini- mum.	Monthly Temper- atures.	Mean Maxi- mum.	Mean Mini- mum.	Monthly Temper- atures.
	"	"	°	°	°	°	°	°
January....	5.54	6.41	85.1	59.1	72.4	84.8	61.2	70.3
February....	6.58	12.76	84.3	60.2	72.3	85.0	62.0	73.5
March.....	3.17	9.62	79.6	56.0	67.8	76.6	57.6	67.1
April.....	2.29	1.12	76.3	51.2	63.8	79.5	55.4	67.4
May.....	0.10	0.97	72.8	40.9	56.9	76.4	45.7	61.0
June.....	0.00	0.72	69.1	35.0	52.1	75.8	39.6	57.7
July.....	0.02	0.14	71.6	35.2	53.4	74.5	38.7	56.6
August.....	1.03	0.04	74.3	40.8	57.6	77.8	49.2	63.5
September..	0.13	1.49	78.8	49.0	63.9	80.7	49.0	64.9
October.....	4.40	4.10	80.9	51.9	66.4	77.1	52.8	64.9
November..	1.78	2.82	84.1	52.1	68.1	81.0	56.8	68.9
December...	3.04	3.52	90.3	59.9	75.1	83.5	59.4	71.4

Onderstepoort is 4300 feet above sea-level, and is situated on a plain about six miles north of Pretoria.

Pietermaritzburg is situated in a valley, and is 2200 feet above sea-level.

In working out the life-cycle the same method was employed as that adopted by Shilston.⁽¹⁾

The ova hatch in two days when placed in contact with the skin, and in three days when placed $\frac{1}{2}$ to 1 mm. from the skin. Eggs which were tied in long wool $1\frac{1}{2}$ inches from the skin were observed to hatch in from six to eight days. The larvae on hatching attack the

(1) Shilston, A. D. Same report, page 72.

skin very quickly and soon commence to grow. After feeding for about thirty-six hours they become quiescent and usually change into nymphae in two days, and always within three.

The nymphal stage lasts from three to four days, and during the first two days of this period they increase rapidly in size and then become quiescent before moulting into adults, the males always appearing a few hours before the pubescent females. After moulting, the adults feed for a short time and then copulation takes place. It was noticed that they usually remained in copulo from twenty-four to twenty-eight hours, and in one case I had a pair under observation that remained in copulo for four days.



G. A. H. Bedford, del.

Fig. 6—*P. communis*, var. *oris* : male.

The pubescent female then changes into an ovigerous female—this she does either during the latter part of copulation or immediately afterwards. After moulting, the ovigerous female commences to feed, and usually starts to lay eggs about twenty-four hours after moulting.

The Vitality of Adults under Abnormal Conditions.

Adults have been removed from sheep and kept in the laboratory under varying conditions to ascertain how long they are capable of retaining their vitality.

1. Twenty females and twenty males were placed in an empty bottle. The males all died in three days, and the majority of females were all dead by the fifth day, but one female lived for ten days.

2. Forty females were placed in an empty bottle. They were all alive on the fifth day, three died on the sixth, thirty-three on the eighth, only three alive on the ninth, and one lived for twelve days.

Large numbers of adults were also placed in manure or in wool and scrapings, with the result that no acari lived for more than ten or eleven days.

The Vitality of the Nymphae and Larvae under Abnormal Conditions.

It was found that the nymphae and larvae do not live so long as the adults off sheep, the majority dying on the second to fourth day after being removed from their host. The longest time nymphae were kept alive for was eight days and larvae three.

The Vitality of the Ova under Abnormal Conditions.

The ova were obtained by placing ovigerous females under cover on a sheep and removing the ova laid about twelve hours after the females were placed on the sheep. For each observation made ten ova were employed, and each observation was repeated several times.

1. Ova placed in an incubator at 37° C. hatched in two days when the atmosphere was moist, but failed to hatch in a dry atmosphere.

2. Ova kept in an ice-chest for ten days and afterwards transferred to an incubator at 34° C. usually hatched on the third day, and a few on the fourth day after being placed in the incubator. Ova placed in the ice-chest for longer periods all failed to hatch.

3. Ova exposed to direct sunlight always shrivelled up in a few hours. However, Shilston⁽¹⁾ records hatching a number of ova in four days under the same conditions.

4. Ova kept at room temperature all hatched in five days in summer, but failed to hatch during the winter months.

5. Ova placed in dry sheep faeces at room temperature all failed to hatch both during the summer and winter months.

Transmission Experiments.

Attempts were made to try and transmit the acari to a goat, horse, and calf. Although large numbers were placed in the ear of a goat on several occasions, and on the neck of a horse and calf, in no case did they become infected.

In one instance a number of acari were found in the ear of a sheep which had just died. In size the acari were larger than those of the var. *ovis*, the pubescent female measuring 0.56 to 0.57 mm. in length, the ovigerous females 0.73 to 0.75 mm., and the males 0.5 mm. Although large numbers of acari were placed in the ears of a goat and sheep the animals did not become infected.

The Length of Time Kraals (Sheep Enclosures) can Remain Infected after the Removal of Scabby Sheep.

It is a common belief in South Africa among the majority of sheep farmers that sheep kraals remain infected for months, and I have even heard it said two years after the removal of sheep infected with *Psoroptes*. Their theory is that the acari can live in the manure or crevices of the kraals for a long period, or that the ova dropping from sheep get buried in the manure, and if by chance these are brought to the surface, they are hatched by the heat from the sun. Therefore, it is a common practice among the farmers in this country to burn their kraals after they have become infected.

(¹) Shilston, A. D. Same report, page 84.

The experiments carried out by Stockman, Shilston, and myself prove that the acari cannot live for any length of time off their natural host; also, I have found that ova placed in direct sunlight shrivel up in an hour or two.

Experiments were carried out at Onderstepoort to ascertain how long kraals could remain infected after the removal of infected sheep. These experiments were carried out as follows:—

Two wire enclosures were put up; each of these measured 25 square yards. In the middle of each enclosure a stone kraal was built measuring 4 square yards and 4 feet in height. In one was placed a large quantity of manure, about two to three feet deep, whereas the other contained no manure.

A large number of infected sheep were placed in these kraals for one month—they were allowed in the enclosures during the day and kept in the stone kraals at night. On the day the sheep were removed from the kraals a hundred acari were collected and placed in the stone kraals in the wool which was found lying about on the ground. This was done to ensure that the kraals were properly infected at the time the scabby sheep were removed. The kraals were then left vacant for a certain number of days; then ten clean sheep were placed in each kraal and kept under close observation to see if they became infected.

The results obtained from these experiments are as follows:—

Experiment No. 1.—Kraal left vacant for eight days after the removal of scabby sheep. *Result:* Three sheep out of the ten became infected.

Experiment No. 2.—Kraal left vacant for nine days after the removal of scabby sheep. *Result:* Three sheep out of the ten became infected.

Experiment No. 3.—Kraal left vacant for ten days after the removal of scabby sheep. *Result:* None of the clean sheep became infected.

Experiment No. 4.—Kraal left vacant for fifteen days after the removal of scabby sheep. *Result:* None of the clean sheep became infected.

Although these experiments show that infected kraals do not remain infected for a longer period than nine days, it would be a safe precaution to recommend farmers not to place clean sheep in a kraal which has previously become infected for about sixteen days after the removal of the scabby sheep.

P. communis, var. *caprae*.

This variety is common in the ears of goats in South Africa, but fortunately it is not such a serious pest as the variety last dealt with, and is much easier to exterminate on account of its habit of only being able to live in the ears. Any of the dips recommended in the paper by me on sheep dips in this report will be found serviceable for dealing with this pest. Before applying the dip to the ears, the crusts formed by the acari should carefully be removed with a knife or any such serviceable instrument.

In appearance the acari resemble those of the var. *ovis*, but they are a little longer in size, and the adults are often brown in colour.

Attempts were made at Onderstepoort to work out the life-cycle on the backs of sheep, but the acari all died when they reached the nymph stage, although Shilston was successful in working out the life-cycle the same way and found it to be the same as in the var. *ovis*.

P. communis, var. *bovis*.

A large number of acari were forwarded to the laboratory by J. P. Venter, Sheep Inspector, Kroonstad, Orange Free State, taken from an ox on the 10th September, 1912. In April, 1915, we received a piece of hide taken from an ox at Lydenburg which was infected with *P. communis*, var. *bovis*, and *S. scabiei*, var. *bovis*. The *Psoroptes* were living in the thick crusts formed by the latter species. These are the only records I know of this variety having been found in South Africa.

P. communis, var. *equi*.

I know of no record of this variety having been found in South Africa.

P. communis, var. *cuniculi*.

This variety is very common in the ears of domestic rabbits in South Africa.

In appearance the acari resemble those of the var. *caprae*. In colour the adults are usually brown to dark-brown, and I have even seen them black.

Attempts were made to try and transmit the acari to a horse, calf, and sheep. Although a large number of acari were placed on each of these animals in no case did they become infected.

Horaëus ⁽¹⁾ was also unsuccessful in transmitting this variety to horses and sheep, and had the same results with dogs and cats.

The following are the facts published by Caying ⁽²⁾:—A horse contracted very extensive scabies in a stable where there was a hutch full of rabbits. These were removed and the disease disappeared after a few days' treatment. With another horse the affection was localized on the points where the pad and other articles of harness rested on the skin; there were also some disseminated patches. The harness in question was usually laid on a hutch containing rabbits affected with psoroptic scabies. When the latter were taken away and the stable disinfected the malady was speedily cured. Cadeac remarked at the Toulouse Veterinary School that rabbits became affected with auricular scabies when horses suffering from psoroptic scabies were introduced into the stable in which their cage was kept.

In eradicating this pest, the same remarks apply here as previously mentioned for dealing with the var. *caprae*.

⁽¹⁾ See Neumann's Parasites, 2nd edition, page 624.

⁽²⁾ *Ibid*.

Fig. 1.



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Psoroptes communis, var. *ovis* : ova.

Fig. 7.





**THE
SULPHUR SHEEP DIPS.**

BY

HENRY H. GREEN,

Biochemist, Division of Veterinary Research.

The Sulphur Sheep Dips.

HENRY H. GREEN, Biochemist, Division of Veterinary Research.

THE so-called "dip controversy" is already so familiar to the South African public that no explanation need be offered in presenting a contribution to the study of those dips in most general use by the sheep inspectors of the Union.

The general attitude of the Union Government towards the eradication of scab is also well known. Provided farmers keep their flocks clean, they are at liberty to do so by the use of whatsoever dip they please, either home-made or proprietary. Wherever a specific order for compulsory dipping is found necessary, the sheep owner is again allowed to use his own discretion in the selection of a suitable dip, provided that he is regarded as likely to carry out the cleansing of his infected flock satisfactorily. If, however, any doubt exists as to the probable thoroughness with which the eradication of scab is likely to be performed, the execution of the dipping order is undertaken by an official inspector of the Division of Sheep. The inspector then selects the dip, and since it is highly desirable that the practice of different inspectors in different parts of the country should be uniform, more particularly in view of possible compensation claims made against the Government for loss of sheep in the hands of an inspector, the Sheep Division have issued recommendations concerning the dips to be used.

The instructions to use home-made "caustic soda and sulphur" or "lime and sulphur" preparations arose not from any desire to expressly discountenance the use of proprietary dips, but from the desire to use preparations whose ingredients were known and whose efficacy could be relied upon, and to avoid favouring one proprietary article to the detriment of another.

The departmental recommendations, published in pamphlet form and accessible to the farmer, did, of course, have the effect of encouraging the general use of home-made rather than proprietary dips, and in consequence gave rise to a controversy concerning the efficacy of different dips for the cure of scab and the effect of different dips upon the wool and skin of the sheep. The criticism levelled at the Sheep Division was doubtless in part disinterested. It was, however, also in some cases part of the advertising propaganda of enterprising manufacturers of proprietary preparations, as for instance, in the case of the anonymous pamphlet circulated throughout the Union in the early months of 1914 to which Mr. G. B. L. Enslin, Chief of the Division, found it necessary to reply.¹ Much of the criticism passed from time to time was *a priori* absurd, involving as it did a complete ignorance of the chemical composition of the dips recommended. At the same time it was desirable that a general survey of the dips most commonly used in South Africa should be made, and at the request of Mr. Enslin the question was taken up by the Director of Veterinary Research, who in turn instructed the present writer to

¹ *Farmers' Weekly*, 17th June, 1914, p. 1794.

report upon the general properties of the sulphide dips. To Mr. Bedford was assigned the work of reinvestigating the life-history of the scab parasite and of conducting dipping trials with various proprietary preparations. Bedford's report, as also the earlier work of Mr. Shilston (formerly of this Division) is included in another place in the present issue of the Report of the Director of Veterinary Research.

The chemical aspect of the sulphide dips to be dealt with here may be taken in the order:—

1. Caustic soda and sulphur dip.
2. Lime and sulphur dip.
3. Loogas and sulphur dip.
4. Efficacy for the cure of scab.
5. Probable decomposition of the sulphide dips in the fleeces of the sheep.
6. Effect upon the skin and wool of sheep.
7. Summary.

1. THE CAUSTIC SODA AND SULPHUR DIP.

According to the recommendations of the Sheep Division in 1913,² the mode of preparation advised is to mix 20 lb. of sulphur into a thin cream with not more than $2\frac{1}{2}$ gallons of hot water and then to slowly sprinkle in 5 lb. of caustic soda with constant stirring. After 40 minutes the resulting mixture is poured into 100 gallons of water for tank use. In the original directions it was recommended *not* to boil the mixture but in the revised directions³ boiling is recommended, not as necessary, but rather as advisable as a mode of ensuring complete combination of the caustic soda and sulphur in cases where preliminary mixing has been improperly carried out. As we will see later, this point is immaterial.

The use of "flowers of sulphur" was recommended in preference to the somewhat cheaper "ground rock sulphur" in view of the contention of Haslam⁴ that the latter article is unsuitable. As will be shown presently, the distinction is a purely academic one and of no practical significance.

From the mode of preparation one would, in accordance with ordinary textbook teaching, *a priori* expect the reaction to result in the formation of sodium thiosulphate and one or more of the higher sulphides of sodium, in accordance with the equation:—



If $x=12$, then sodium pentasulphide Na_2S_5 , the highest sulphide usually regarded as capable of formation, could be formed, and since the proportions $6\text{NaOH} : 12\text{S}$ correspond to a weight ratio of 240 caustic soda : 384 sulphur, it is seen that the proportions recommended for making the dip (5 : 20) allow not only of the formation of a pentasulphide, but leave a large excess of sulphur unacted upon.

Reference to the last edition of any of the standard chemical dictionaries gives five sulphides of sodium as possibly existing, the monosulphide Na_2S , the disulphide Na_2S_2 , the trisulphide Na_2S_3 , the

² Enslin : Scab in Sheep and Goats, Bulletin No. 3, Department of Agriculture, Union of South Africa.

³ Enslin : *Agricultural Journal of the Union of South Africa*, July, 1914.

⁴ Mallinson : Effect of Dips on Wool, *Agricultural Journal*, Union of South Africa, October, 1913.

tetrasulphide Na_2S_4 , and the pentasulphide Na_2S_5 , the existence of this last being regarded as doubtful (Schöne, Sabatier, Geuther, Chapman Jones).

All five may be theoretically supposed to exist, along with the products of partial hydrolysis, in the aqueous liquid obtained by the reaction between caustic soda and sulphur in hot solution.

In addition to these, other polysulphides such as the tetrasodium monosulphide Na_4S_9 , a hydrate of which was described by Bloxam, have been supposed to exist, but their existence as truly independent entities appears very doubtful. Various hydrosulphides, hydroxy-sulphides, and oxysulphides may also be supposed to occur, and there is very little doubt that the hydrosulphide NaHS definitely exists, in traces at least, as an hydrolytic product of the higher polysulphides in solution.

More recently Rule and Thomas,⁵ by the reaction of varying quantities of sulphur upon alcoholic solutions of sodium hydrosulphide, have obtained the disulphide and tetrasulphide in the pure condition, indicating these at least as stable polysulphides. Although polysulphides higher than the tetrasulphide were not isolated in the pure state, evidence is adduced to show that they exist in alcoholic solution, and sulphides even higher than the pentasulphide are conjectured.

The complexity in the compounds of sodium and sulphur has led to a great deal of confusion on the part of the writers involved in the "dip controversy," and indeed the most persistent Bradford critics of the recommendations of the Sheep Division go so far as to insist that the resultant dip consists mainly of ordinary sodium sulphide (Na_2S) [*vide* "The Wool Record," 26th March, 1914], which in solution hydrolyses to give free caustic soda and the powerfully depilatory hydrosulphide. Such a contention is of course false. It would never have been made by any one really familiar with the dip, and an analysis at once shows the fallacy. The following percentage figures represent the composition of a sample of dip carefully made according to recommendation, using hot water, but refraining from boiling by the application of external heat. The data represent the actual composition of the dip at tank dilution:—

Total sodium	0.287
Sodium as thiosulphate... ..	0.103
Sodium as sulphate and sulphite	0.006
Sodium as sulphide and polysulphide	0.178
Total sulphur	0.791
Sulphur as thiosulphate	0.143
Sulphur as sulphate and sulphite... ..	0.004
Sulphur as sulphide and polysulphide	0.644

Sodium was estimated by conversion into sulphate, thiosulphate by iodine in the filtrate after precipitating sulphides with ammoniacal zinc chloride, sulphate and sulphite by barium chloride after conversion of thiosulphate to tetrathionate. Total sulphur was determined by barium chloride after oxidation with hydrogen peroxide in alkaline solution, and the polysulphide sulphur was checked by oxidation of the ammoniacal zinc precipitate. A discussion of analytical methods is given in a separate paper in the present report and need not therefore be entered into here.

⁵ Rule and Thomas: The Polysulphides of the Alkali Metals, *J. C. S.*, DCXV, January, 1914.

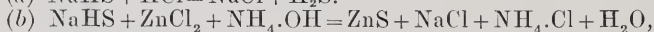
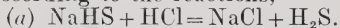
From the data given it is seen that the amount of sulphur in any form other than thiosulphate and polysulphide is negligibly small.

The dip was found to be almost neutral to phenol phthalein. The pink colour formed on addition of this indicator at once faded on the addition of a few drops of $N/_{10}$ acid (per 10 c.c. of solution), returning again within a few seconds and again fading on further addition of small quantities of acid. This points to the absence of free caustic soda since free hydroxide titrates quantitatively to this indicator without fading of colour. The presence of sodium monosulphide in any considerable quantity is also negated by the behaviour towards phenol phthalein since that salt registers half its base on acid titration to this indicator. The precise end-point to phenol phthalein could not be obtained owing to slow progressive hydrolysis of polysulphide, but the behaviour was sufficiently marked to establish the absence of either free alkali or alkaline monosulphide in anything but very small amount.

On precipitating the sulphides present by a solution of zinc chloride containing excess of neutral ammonium chloride, and standard in respect to free ammonia, no appreciable increase in alkalinity could be detected in the filtrate and no appreciable free base could therefore be present.

To methyl orange, which is not sensitive to sulphuretted hydrogen, the dip is of course markedly alkaline, and on titration against $N/_{10}$ acid to this indicator a figure was obtained closely corresponding to the titration value obtained with $N/_{10}$ ammoniacal zinc chloride, i.e. to the "monosulphide index" or "base in combination as sulphide and polysulphide." This fact also precludes the presence of anything more than a trace of free caustic soda, and at the same time indicates the absence of free hydrosulphide (i.e. hydrosulphide not in hydrolytic equilibrium with hydroxide).

According to the reactions,



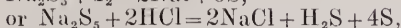
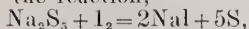
the zinc method (in presence of excess of ammonium chloride and ammonia) would register twice as high a titer as the acid titration to methyl orange would do, if the base present were in the form of hydrosulphide. Taking 20 c.c. of dip the actual data were 15.3 c.c. $N/_{10}$ am. zinc and 15.5 c.c. $N/_{10}$ acid. The slightly higher acid figure may be explained either on the assumption of a trace of free hydroxide or on the assumption of a trace of sodium carbonate derived from the hydroxide used.

Taking the behaviour of the dip towards phenol phthalein, methyl orange, and ammoniacal zinc, into full consideration, we may for practical purposes ignore all bodies other than thiosulphate and polysulphide.

From the analysis already given we have sodium 0.178 per cent. in simple union with sulphur 0.644 per cent. This gives a ratio, sodium: sulphur: : 1: 3.62, which is higher than that required for the pentasulphide and corresponds to a formula approximately $\text{Na}_2\text{S}_{5.2}$. This unexpected result either indicates the existence of sulphur in physical solution or predicates the presence of a polysulphide higher than the pentasulphide, which by admixture raises the atomic ratio above 2: 5. That this figure (5.2) does not represent the limit of sulphur capable of being held in solution, is indicated by the fact

that on titration of the dip with acid or iodine, liberation of sulphur is not immediate but is appreciably delayed.

According to the reaction,



precipitation of sulphur should take place at once.

If, however, the iodine be added drop by drop to the dip already containing sulphur in solution past the point represented by the pentasulphide, the sulphur precipitated in the area of local excess is *redissolved* on shaking, and more especially on heating. An attempt was made to determine the amount of sulphur which could be redissolved, by noting the point at which permanent precipitation began, and calculating the sulphur which should theoretically be precipitated by the iodine (or acid) used. No sharp end-point could, however, be determined. As the point of permanent precipitation approached, it appeared as if part of the sulphur redissolved even after marked opalescence became visible, and we therefore contented ourselves with noting that sulphur could be held, either in combination or in solution, at a point well beyond the proportions required for the pentasulphide.

The presence of sulphur in solution above the quantity required for the formation of a pentasulphide has been verified on over half a dozen samples of dip. On standing out of contact of air for any length of time, however, a tendency is shown for the excess of sulphur to deposit. The deposition is usually slow and may take weeks. In concentrated solutions of the dip the deposited sulphur is usually crystalline. Although the ratio of sodium to sulphur (after allowing for the thiosulphate present) is commonly above that required for the pentasulphide, there is no direct proof that the pentasulphide exists as such in solution. The solution may be supposed to consist of any mixture of polysulphides above and below the pentasulphide, or to contain a lower stable sulphide holding sulphur in physical solution. This latter supposition did not appear probable to us, and in any case the distinction between a pentasulphide and, let us say, a disulphide with three equivalents of sulphur in solution, would be difficult to make. The significance of the distinction would depend upon the stability of the higher polysulphides, which are generally regarded as containing their sulphur in very loose combination. In working with dips made up under varying conditions we were, however, convinced of the marked tendency towards the formation of higher polysulphides rather than lower, even where the lower sulphides might reasonably have been expected. An attempt was therefore made to compare the behaviour of sulphur dissolved in the dip with that of free sulphur in suspension. For this purpose a sample of dip was boiled (*a*) in presence of free caustic soda, (*b*) in presence of free caustic soda and free sulphur. The "monosulphide index" was determined before and after treatment, ammonium chloride being used to counteract the possible influence of residual caustic soda upon the accuracy of the ammoniacal zinc titration. The protocols are included in the accompanying paper already referred to, but the main result may be mentioned here.

Commencing with a solution in which the ratio of sodium to sulphur after allowing for thiosulphate corresponded almost exactly to a pentasulphide ($\text{Na}_2 : \text{S}_{5.04}$) it was found that forty minutes' gentle boiling with sufficient caustic soda to theoretically reduce to below

the disulphide, failed to bring the ratio down to that corresponding to the tetrasulphide. The actual ratio $\text{Na}_2\text{S}_{4.2}$ found in one case corresponds to a mixture of pentasulphide with tetrasulphide. As determined from the alkalinity of the filtrate from the zinc precipitate, using known excess of ammonia and ammonium chloride, about 84 per cent. of the added caustic soda *remained free*. On the other hand when the same quantity of dip, after addition of caustic soda exactly as before, was boiled for only fifteen minutes with excess of free sulphur added as an aqueous paste, it was found that the added caustic soda was almost quantitatively converted into further polysulphide and thiosulphate.

The sulphur present in the dip in excess of, say, the disulphide, does therefore *not* behave as free sulphur, and polysulphides higher than the tetra are capable of existing in presence of *free caustic soda in hot solution*. Free caustic soda should react with sulphur in "physical solution" much more readily than with powdered roll sulphur, and the apparent presence of residual pentasulphide after forty minutes' heating with free alkali points not only to the absence of dissolved sulphur, but also to a remarkable degree of stability of the higher polysulphides.

From this evidence we feel thoroughly justified in concluding that the pentasulphide is the dominant constituent in the dip, and that, apart from the concomitant thiosulphate, other derivatives are not present in appreciable amount. Where, as already mentioned, the sulphur exceeds that actually required for the existence of a pentasulphide we are still at liberty to regard the excess as in "solution." The tendency for such excess sulphur to deposit or crystallize out of strong solution on prolonged standing, and the fact that such excess seems to combine readily on further addition of free caustic soda, may be taken in support of this idea. On the other hand, the great extent to which a concentrated solution, containing sulphur in excess of the polysulphide proportion, can be diluted with air-free distilled water without precipitation of sulphur, and the apparent ease with which a dilute solution already containing sulphur in the pentasulphide ratio can redissolve part of the sulphur precipitated by iodine or acid, argues rather against the idea of simple solution and in favour of the existence of polysulphides even higher than Na_2S_5 , or possibly of hydropolysulphides $\text{Na}(\text{HS}_x)_2$.

There can be little doubt that the *main* reaction occurring when caustic soda combines with excess of sulphur in hot solution, is represented by the equation:



In practice a trace of sulphate is formed and the thiosulphate is always somewhat higher than that indicated by the equation. The secondary reaction involving increase of thiosulphate is not quite clear, but is probably due either to atmospheric oxidation or to recombination of further free sulphur with alkali derived by dissociation of polysulphide,

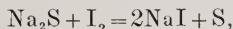


Any escaping sulphuretted hydrogen would disturb the equilibrium and allow of continued primary reaction of sulphur with the reformed caustic soda. That this explanation is a probable one is indicated by the fact that the thiosulphate slowly increases at the expense of the pentasulphide when the dip is boiled for any length of time.

In so far as the intermediate steps in the main reactions are concerned, little can be said. It may be that monosulphide is first formed, and that this then combines with more sulphur to produce higher sulphides. If this be the case, it is certain that the second reaction is much faster than the first, since even when the caustic soda used is initially in large excess of the sulphur the monosulphide does not remain in solution. 100 grammes of caustic soda boiled with 20 grammes of sulphur at a bulk of 500 c.c. gave a mixture of sodium thiosulphate, sodium polysulphide with an atomic ratio $\text{Na} : \text{S} : : 2 : 3.9$, and large excess of residual free caustic soda. The close approximation of the sulphur-base ratio to that required for the tetrasulphide is suggestive. Where the caustic soda is in large excess of the sulphur, the tendency is towards the formation of tetrasulphide, and not, as might be expected, towards the mono-, di-, or trisulphide. Where the sulphur is in large excess of the caustic soda the tendency seems to be towards the formation of pentasulphide mixed either with a still higher sulphide or with "dissolved sulphur."

As already indicated, the pentasulphide *once formed* is stable, and appears to be only slowly reduced towards the tetrasulphide by prolonged heating with freshly added caustic soda.

That the building up of polysulphides from monosulphide occurs very easily is shown by the behaviour of solutions of the monosulphide towards iodine. Even in very cold solution, and at a concentration much below $\text{N}/_{100}$, the addition of iodine is accompanied by the immediate formation of polysulphide. The sulphur first deposited at the point of local excess, in accordance with the equation,



is immediately redissolved, with formation of intensely coloured polysulphide.

The reaction between caustic soda and free sulphur is, on the other hand, negligible in cold solution, and sulphur may be stirred in comparatively strong alkaline solution for hours without visible reaction taking place.

Variations in the mode of preparation of the dip.

Under this sub-heading we may consider:—

- (a) The influence of temperature.
- (b) The influence of boiling at varying bulk.
- (c) The influence of the kind of sulphur used.
- (d) The influence of the quantity of sulphur used.

(a) Influence of temperature.

Although hot water is recommended for use in dip-making, it often happens that the recommendation is not closely observed. It was therefore thought of interest to determine the extent of combination taking place at varying temperatures. For this purpose the thermal reaction between caustic soda and water was eliminated by using caustic soda in solution. Twenty grammes of sulphur was worked into a thick homogeneous paste with the minimum of water in a mortar, then transferred to a small Erlenmeyer flask and washed in so that the total bulk of water used was approximately 15 c.c. Ten c.c. of 50 per cent. caustic soda, equal to 5 grammes of solid hydroxide, was then added. These proportions represent those used in actual dip-making. The flask was then immersed in a beaker of

water kept between assigned limits of temperature, and the reaction allowed to proceed with more or less constant agitation for forty minutes. The resulting dip was then diluted, filtered from excess of sulphur, washed up to 1000 c.c., and immediately analysed.

The following Table I indicates the composition of preparations at varying temperatures, any slight errors of dilution being corrected to standard sodium hydroxide content of 0.5 per cent. R and F are duplicates with different samples of sulphur, R being "Capex ground rock," and F being "Brandram's flowers."

Table I.
TEMPERATURE.

	°C. 20-25		°C. 35-40		°C. 50-55		°C. 65		°C. 80		°C. 100	
	R.	F.	R.	F.	R.	F.	R.	F.	R.	F.	R.	F.
Sodium stated as NaOH												
used... ..	·500	·500	·500	·500	·500	·500	·500	·500	·500	·500	·500	·500
Total sulphur in solution	·021	·241	·253	·766	·771	·800	·807	·820	·803	·830	·825	·825
Sulphur as polysulphide	·017	·192	·201	·631	·630	·659	·666	·680	·668	·682	·677	·677
Sulphur as thiosulphate	(·004)	·049	·052	·135	·141	·141	·141	·140	·135	·147	·147	·147

It will be seen from these data that the reaction is very slow in the cold, being almost negligible at 20°-25° C. At this temperature only a trace of polysulphide is formed, and the bulk of the caustic soda remains in the free state. At blood heat, however, the reaction is quite marked, and although very slow at first a considerable amount of polysulphide is formed in forty minutes. The ratio of "monosulphide sulphur" as determined by titration with ammoniacal zinc, to "total sulphide sulphur" as determined by difference from total sulphur and thiosulphate sulphur (ignoring the mere trace of sulphate and sulphite) was found to be 2:5.21. This ratio, indicating the presence of a polysulphide above Na_2S_3 , forcibly illustrates what has already been said in regard to the tendency towards formation of higher polysulphides rather than lower. At the low temperature of 35°-40° C. the primary reaction between sulphur and the hydroxide is slow, but the secondary reaction between the lower sulphides (presumably first formed) and sulphur is rapid. The resulting polysulphide is therefore high, in spite of the fact that the bulk of the caustic soda remains free in solution.

At 50°-55° C. the reaction is comparatively rapid, and within forty minutes is almost complete. Above this temperature the increase in the amount of sulphur dissolved is small.

The amount of free caustic soda remaining in the dip when the reaction takes place above 55° C. is very small, and although it could not be accurately estimated owing to the presence of small amounts of carbonate it may be regarded as negligible.

In almost every case the sulphur in solution indicates the presence either of a polysulphide higher than Na_2S_3 or of small amounts of free sulphur in physical solution.

The results at low temperatures are of particular interest and indicate that under all conditions where sulphur is in excess the reaction takes place along the same lines—the higher temperatures

being necessary only for the elimination of the free caustic soda within limited time.

The more practical test involving the use of cold water in making the sulphur paste, and the raising of the temperature simply by the addition of the caustic soda in solid form, was also carried out. 20 lb. of sulphur was worked into a thin paste by the gradual addition of cold water and constant kneading with the hands. The remainder of the recommended $2\frac{1}{2}$ gallons of water was added and the mixture stirred to a thin paste. 5 lb. of caustic soda was then slowly sprinkled in. About one-half was added within three minutes, and the other half stirred in within the ensuing five minutes. The mixture was constantly stirred for another fifteen minutes and then left to stand, with occasional mixing up, for a further twenty minutes. The initial temperature of the paste was 20°C . On adding the caustic soda this steadily rose to 56°C ., and then gradually cooled off at the rate of about 3°C . every five minutes. The final temperature at the end of forty minutes was 38°C .

The resulting fluid showed the characteristic deep red colour of a well-made concentrated dip. A portion of this was filtered while still warm. On dilution to tank strength the following percentage analysis was shown:—

Total sodium	0.287
(Equivalent to NaOH used)	0.500)
Sodium present as NaOH + Na_2CO_3	0.010
Total sulphur	0.800
Monosulphur equivalent	0.122
Total sulphide sulphur	0.658
Sulphate and sulphite	0.004
Thiosulphate sulphur	0.138
Monosulphide S : total sulphide S = 0.122 : 0.658 = 1 : 5.39.	

The amount of alkali remaining free in the dip is small, and very probably due almost wholly to carbonate. Excluding thiosulphate and sulphate, the ratio of base to dissolved sulphur is well above that required for the pentasulphide. A portion of the concentrated filtered dip was filled into a bottle, sealed, and stored for a week. At the end of this time a considerable quantity of sulphur had crystallized out on the bottom and walls of the bottle, and a second analysis now showed an amount of sulphur in solution corresponding exactly to the pentasulphide.

This experiment shows that it is *possible* to prepare a dip of correct composition even if the water used be cold and the temperature required for the reaction be produced solely by the addition of the caustic soda itself. It must, however, be emphasized that to secure this result the sulphur and water paste must be thoroughly homogeneous, and the quantity of water strictly limited. If these conditions are not observed the sulphur floats on the surface and is not properly acted upon by the caustic soda at the comparatively low resulting temperature.

The effect of boiling the mixture was also tested. In these trials the sulphur was worked into a paste as usual, the caustic soda then rapidly sprinkled in, and the whole boiled for fifteen minutes. The analyses of the resulting dips are not worthy of special comment, since they were practically identical with that of the dip prepared at the boiling point itself (100°C ., Table I), except that a general

tendency towards slight increase in the thiosulphate was observed. Fifteen minutes' boiling was found quite sufficient to complete the reaction, and more prolonged boiling merely resulted in slow increase of the quantity of thiosulphate present, with concomitant reduction in the amount of sodium present as polysulphide. Boiling, then, is quite unnecessary, provided that the sulphur used is first worked into an homogenous paste. In any case the recommendations of the Sheep Division stipulate the use of hot water in making the paste, and this incidentally involves subsequent boiling by heat of reaction as the caustic soda is sprinkled in—or at least maintenance at the boiling point for the period during which the caustic soda is being added. Boiling would therefore only be advised as a safeguard for completing the combination in those cases where the preliminary emulsification of the sulphur had been imperfectly performed. Even then the safeguard cannot wholly be relied upon, since if the preliminary mixing of the sulphur and water is very badly done the finely divided sulphur is not properly wetted, and for the most part floats upon the surface during the subsequent boiling—out of reach of the hot caustic soda solution.

The thorough wetting of the sulphur is the most important consideration.

(b) Influence of Boiling at Varying Bulk.

If boiling by the application of external heat be resorted to, the necessity for limiting the quantity of water used disappears. It is now no longer necessary that the water be kept within $2\frac{1}{2}$ gallons, since the maintenance of temperature is no longer dependent upon the heat of solution of the caustic soda.

The following analyses of dips prepared by boiling at varying bulk illustrate this point (Table II). The sulphur was, as usual, mixed to a paste with a little water. The requisite total bulk of water was added and the caustic soda stirred in. The fluid was then boiled for fifteen minutes and diluted to tank strength. In the analyses the traces of sulphate and sulphite present were ignored and total sulphide sulphur is simply taken as the difference between total sulphur and thiosulphate sulphur.

Table II.

Analysis.	Quantity of water used in gallons.			
	$2\frac{1}{2}$	5	10	20
Total sodium stated as caustic soda used	0.500	0.500	0.500	0.500
Total sulphur	0.825	0.825	0.820	0.799
Total sulphide sulphur... ..	0.677	0.678	0.663	0.632
Sulphur as thiosulphate	0.147	0.147	0.157	0.167

In no case could any residual free caustic soda be detected. It will be noted that, beyond a slight increase in the amount of thiosulphate and concomitant small reduction in the amount of polysulphide, the composition of the dip is not affected by the volume of water used in boiling—within fairly wide limits. If, therefore, the maker of the dip elects to boil the mixture he may be guided in his quantities simply by the size of boiling pot which he happens to have at his disposal.

(c) *Influence of the kind of Sulphur used.*

The two forms of sulphur which come into consideration are "flowers of sulphur" and "ground rock sulphur." The relative value of these two forms of sulphur was investigated at the express request of Mr. Enslin, who, acting upon the generally accepted opinion that "flowers" was superior to "ground rock," recommended its preferential use. Certain firms supplying *finely ground* rock sulphur took exception to this, and more particularly to the exclusive recommendation of "flowers" in Mr. Mallinson's report (*l.c.* 4), and claimed that no material difference existed between the two forms of sulphur.

Since there is evidently some misconception in the lay mind as to the distinction between the two forms it is perhaps worth while reminding the general reader of the nature of the difference—as expounded in the textbooks. In the purification of sulphur by the distillation of the crude material the vapours passing over may be condensed (*a*) suddenly on the cool walls of the receiving chambers (below 100° C), and (*b*) in liquid form in warm chambers or after the condensing chambers have become sufficiently heated to prevent rapid solidification. In the first case the resulting product consists of a fine powder which, owing to its sudden condensation, has passed through the liquid phase without complete crystallization. This is "flowers of sulphur," and genuine flowers should, when freshly prepared, contain 33 per cent. or more of the amorphous optically isotropic modification, although some varieties, more correctly termed "sublimed sulphur," may only contain 5 to 6 per cent. Owing to its mode of preparation it is globular instead of crystalline in microscopical appearance. In the second case the molten sulphur collecting at the bottom of the chamber is run off into moulds, and on solidification is known as "roll sulphur" or "rock sulphur." This may then be milled and marketed as "ground rock sulphur" or "sulphur flour" in the form of a very fine powder very like flowers of sulphur, except that it is a little paler in colour. Microscopically it is crystalline in structure, and shows up as minute sharp-edged fragments. The difference in microscopical appearance is well shown in the photographs of Plate 1.

Although there is this apparent difference in form there is no real distinction of kind, because in a comparatively short time the amorphous variety passes spontaneously over into the more stable crystalline form, retaining merely its externally globular shape. The result is that, except for the mere shape, "flowers of sulphur" and "ground rock sulphur" are identical.

The practical difference between the two forms in regard to suitability for dip-making therefore depends upon (*a*) any differences in the extent of surface exposed to the caustic soda for solution, and (*b*) any differences which may affect the ease of securing homogeneous mixing of the sulphur and water.

In regard to the first point it may be stated that a *finely ground* sample of rock sulphur exposes so large a surface for solution that there is no difficulty in the way of rapid reaction with the caustic soda, and that in any case the natural cohesion of the finely divided particles obliterates minor differences in solution surface. In respect to the second point, it is found in practice that flowers of sulphur, which is often coarser than finely ground rock sulphur (as determined by the channel test and by microscopical appearance) is more easily

wetted, and therefore more easily mixed into a cream with water in preparation for the addition of the caustic soda. A little extra trouble, however, secures the thorough wetting of rock sulphur and a creamy consistency of the aqueous mixture, so that no real objection can be urged on this score.

Theoretical considerations thus lead to the assumption that the two forms of sulphur are equally suitable for dip-making, and, as the following data show, the assumption is justified in practice. The analyses represent two strictly comparable dips made up on the practical 100-gallon scale, according to the original recommendations of the Sheep Division:—

	Brandram's "Flowers."	Capex "Ground Rock."
Sodium stated as NaOH	0.500	0.500
Total sulphur	0.741	0.742
Total sulphide sulphur... ..	0.606	0.610
Thiosulphate sulphur	0.135	0.132
Sulphate and sulphite	Trace	Trace
Free caustic soda	Nil	Nil

It will at once be seen that there is no difference between the two dips. The data of the previous Table I, in which the duplicates were carried out with the two forms of sulphur for the sake of more extensive comparison, indicate the same identity. This equality of course only holds when the rock sulphur is finely ground. Coarsely ground sulphur is only slowly acted upon by caustic soda even on relatively long boiling, and is thus quite unsuitable for dip-making. In purchasing this brand, some guarantee of fineness of grinding should therefore always be demanded, and in this respect a specification of 60°-65° chancel is not unreasonable. Most samples of sulphur on the South African market come up to this standard and show a degree of fineness which entitles them to the designation of "sulphur flour" in distinction to the less specific name of "ground sulphur." We have only seen one really coarse sample of ground sulphur. This specimen was sold in the form of a "meal," and was therefore quite unsuitable for the preparation of home-made dip.

The average local vendor apparently draws no distinction between "flowers of sulphur" and "sulphur flour." Of fifty samples purchased for dip-making, and sent in for examination to this laboratory by sheep inspectors throughout the Union, eighteen were found to be true "flowers," thirty were finely ground rock sulphur, while two were mixed flowers and rock. Except in a few isolated cases, however, all were sold as flowers of sulphur. Since all fifty samples were sufficiently finely ground for dip-making, the confusion was of no practical consequence. At the same time there is a considerable difference in price between the two commodities, and a distinction should therefore be made by the vendor.

(d) Influence of Quantity of Sulphur used.

In the analyses hitherto given, only dissolved sulphur is taken into consideration. In all cases, rather more than half of the sulphur used in making the dip remained undissolved and was filtered off before making the analysis. Although in practice this sulphur is mixed up in the dipping tank, and if uneaked, as in the case of a well-made dip, is largely retained in suspension by the stirring

incident to the passing through of the sheep, it is questionable whether it serves any useful purpose. The particles of this undissolved sulphur, although comparatively fine, are still coarse enough to be filtered off by the wool of the sheep. The major portion is carried off, irregularly distributed in the fleeces, by those sheep which are first sent through the tank. The sheep dipped last get a much smaller proportion than those dipped first. It is probable, however, that this irregularly distributed free sulphur is more or less inert and functionless in comparison with the exceedingly finely divided and evenly distributed sulphur which is precipitated by the subsequent decomposition of the dissolved polysulphide sulphur on the backs of the sheep.

If the undissolved original sulphur be regarded as useless, it is obviously uneconomical to use so much. From the analyses already given it is seen that the 5 lb. of caustic soda used is only capable of combining with about 8 lb. out of the 20 lb. of sulphur taken. Allowing a reasonable working margin, it appears that 10 lb. of sulphur should be ample. If this quantity be adopted, however, special care must be taken to ensure a very thorough preliminary wetting of the sulphur, and mixing into an homogeneous cream with water, before the caustic soda is added. Any sulphur unwetted in the preliminary stage tends to float on the surface in spite of subsequent stirring and thus to escape reaction. Furthermore, the particles of sulphur are never absolutely uniform, and the larger fragments tend to settle out, or to be attacked only very slowly, even when stirred up. Where only 10 lb. of sulphur is used, the margin of excess is small, and any sulphur escaping combination tends to leave a corresponding amount of free caustic soda in the resultant dip. Where 20 lb. of sulphur is used, the margin of excess is so large that the reaction with caustic soda is completed in spite of minor imperfections in the preliminary paste. A few trials were conducted, using 10 lb. of sulphur instead of 20 lb., and it was found that at the usual working bulk of $2\frac{1}{2}$ gallons, and under the usual working conditions, the tendency always was towards the presence of small amounts of free caustic soda in the resultant dip. If, however, the original cream was made with extreme care and the caustic soda evenly sprinkled in with constant stirring so as to avoid local caking of sulphur, the resultant dip was found to be free from caustic soda and contain the normal amount of dissolved sulphur. In those cases in which the dip was found to be imperfect, fifteen minutes' subsequent boiling, with regular stirring in of floating sulphur, sufficed to remove the residual alkalinity.

The following analyses offer comparison between two dips, one made up with 10 lb. of sulphur and the other with 20 lb., 15 minutes' boiling being given in each case. "Capex" ground rock sulphur was used at a boiling bulk of 5 gallons. Portions of the concentrated dips formed were then filtered while still fairly hot, diluted to tank strength and analysed, any slight amount of free sulphur liberated on dilution being included in the "total sulphur" figure as usual.

	Sulphur used.	
	10 lb.	20 lb.
Total sodium expressed as NaOH used...	0.500	0.500
Total sulphur	0.803	0.815
Thiosulphate	0.147	0.147
Free caustic soda	Nil	Nil
Sodium carbonate	Traces derived from NaOH used.	

It is seen that the presence of the extra 10 lb. of sulphur makes no practical difference to the amount of sulphur going into solution. As just stated, however, very thorough mixing of the aqueous sulphur cream is essential if the half quantity of sulphur be used in practice, and especial care must be taken to prevent caking in adding the caustic soda. Fineness of division of the sulphur used is also more important, and the precaution of boiling is advisable. But even under ordinary conditions, the use of 20 lb. of sulphur is unnecessary, since 15 lb. provides an ample margin where ordinary care is adopted. The original recommendations of the Division of Sheep advising 20 lb. of sulphur were probably framed with the idea of allowing a big margin for imperfect mixing.

From time to time analyses have been made of fresh dips drawn from the laboratory dipping tank just before a batch of sheep were put through. These represent dips as they may be expected in field practice, having been made up by the lay officer carrying out the dipping. Analyses were made on the filtered samples. In most cases the total sulphur was found to be slightly lower than in the dips made up in the laboratory, probably as a result of deposition in the tank. The ratio of "monosulphide sulphur" to "total sulphide sulphur" was, however, always very close to 1 : 5.

The acid titration to methyl orange always showed a higher reading than the ammoniacal zinc titration, but the discrepancy rarely exceeded that legitimately attributable to the presence of carbonate derived from the commercial caustic soda used. In no case could any serious amount of free caustic soda be detected.

This section may be concluded with an analysis of a concentrated commercial "caustic soda and sulphur" preparation which is on the South African market under the name "O'Gorman's Liquid Sulphur Dip."

	Drum fluid.	Tank strength. (1 fluid in 30 water).
	Per cent.	Per cent.
Total sodium Na	8.16	0.272
Total sulphur... ..	16.23	0.541
Sulphur as thiosulphate	6.72	0.224
Sulphur as sulphate and sulphite	0.12	0.004
Total sulphide sulphur	9.39	0.313

From the point of view of the total sodium equivalent, the dip at the tank strength is slightly weaker than the home-made preparation—0.272 per cent., equivalent to 0.473 per cent. of caustic soda used in manufacture, as against 0.500 per cent. for the home-made dip. This would be of little consequence but for the fact that so large a proportion of the base is present as thiosulphate. As will be shown presently, thiosulphate has very little value in curing scab, and the efficiency of a dip therefore depends upon its content in polysulphide sulphur. In the preparation under consideration, the total sulphide sulphur at the tank strength recommended is only 0.313, or less than half that of a home-made preparation. To give a dip of efficiency equivalent to that of the home-made article, O'Gorman's fluid would have to be used at more than twice the strength recommended in the directions upon the drum.

Otherwise the dip is permissible, although a much better product could be marketed.

In the present paper we are not concerned with the economics of the question.

2. THE LIME AND SULPHUR DIP.

The consideration of this preparation may be roughly divided:—

- (a) The composition of the dip.
- (b) Conditions controlling the solution of lime and sulphur.
- (c) The quality of lime used in dip-making.
- (d) The quality of sulphur used.
- (e) Commercial lime sulphur concentrates.

(a) *Composition of the Dip.*

According to the recommendations of the Division of Sheep this dip is always made by boiling. 25 lb. of sulphur, and either 20 lb. of slaked lime or 15 lb. of unslaked lime, are taken and mixed thoroughly into a paste with 3 or 4 gallons of water. This is then thrown into a pot containing 25 to 30 gallons of boiling water and boiled for thirty to forty minutes. The contents of the boiling-pot are transferred to a barrel, and after allowing the sediment to settle the clear fluid is run off into the dipping tank from a bung-hole 4 inches from the bottom of the barrel. 100 gallons of water are then added to bring to tank strength, or, alternatively, 70 gallons of water to bring to a total bulk of 100 gallons.

Since this dip is always made by boiling there is no necessity to consider the effect of temperature upon the reaction taking place. In any case combination appears to be slow at temperatures much below the boiling point.

As a general preliminary, the composition of a lime-sulphur dip, as used in one of the laboratory dipping experiments, was determined. 15 lb. of burnt lime and 25 lb. of sulphur had been boiled together for forty minutes at a bulk of 25 gallons, and the concentrated solution made up to 100 gallons in the dipping tank. A portion of this was then filtered and taken for analysis. Calcium was determined by permanganate titration of the oxalate, and sulphur in various forms by the methods already mentioned in connection with the caustic soda and sulphur dip. Total sulphide sulphur is stated as the difference between total sulphur and sulphur as thiosulphate, sulphate, and sulphite. The validity of the analytical methods adopted is discussed in the companion paper already mentioned, and need not, therefore, be dwelt upon at this point.

Analysis.	Per cent.
Calcium stated as CaO	0.934
Total sulphur	1.972
Total sulphide sulphur	1.517
Sulphur as thiosulphate	0.430
Sulphur as sulphate and sulphite	0.025
CaO as sulphide and polysulphide	0.514
CaO as thiosulphate	0.376
CaO as sulphate and sulphite	0.044

Excluding lime and sulphur in the form of thiosulphate and sulphate, we have lime and sulphur present in the ratio of 1:2.95. This corresponds to an atomic ratio of calcium to sulphur of rather over 5.1, thus indicating the pentasulphide as dominant sulphide, with a small admixture either of a polysulphide higher than the pentasulphide or of sulphur in physical solution. As will be shown later, the occurrence

of sulphur in considerable excess of that required for the pentasulphide is the rule rather than the exception, wherever the dip is prepared by boiling with excess of sulphur, and the analysis is conducted within a short time of preparation. As in the case of the caustic soda and sulphur dip, a tendency towards deposition of excess sulphur on standing in the cold is shown, and the general evidence is, therefore, in favour of the assumption that the pentasulphide is the main stable polysulphide in solution.

Ramsay⁶ has argued that the pentasulphide as such does not exist in solution and that aqueous polysulphide mixtures consist chiefly of the disulphide with free sulphur held in physical solution. We regard this view as erroneous, and support our own belief in the existence of pentasulphide upon the fact that whatever proportions of lime and sulphur be boiled together the tendency is always towards the solution of lime and sulphur in proportions corresponding to a polysulphide *higher than the tetrasulphide*, and usually to a pentasulphide. Further, that if *large excess of lime water* be boiled with an aqueous solution of polysulphide in which lime and sulphur are combined in pentasulphide proportions, the high sulphur ratio is only slowly reduced towards the tetrasulphide. The excess of added lime in solution is partly *precipitated out as free lime* on boiling, and partly remains in solution as free lime. If, now, any free sulphur were present in physical solution, it should rapidly react with the added lime to form further quantity of lower polysulphide, and if the disulphide were the stable constituent in the dip the final atomic ratio of calcium to sulphur should be 1 to 2, instead of 1 to over 4, as actually found.

Further discussion on this point may be omitted here, since it is taken up in the companion paper on constitution and methods of analysis.

At this point, however, it may be stated that the close agreement between the total base present in combination in sulphide form as determined by acid titration with methyl orange, and the "monosulphur equivalent" as determined by titration with ammoniacal zinc chloride, indicates the absence of hydrosulphide and of anything more than a trace of free lime.

Towards phenol phthalein the dip behaves very much as did the caustic soda and sulphur dip. The pink colour to this indicator fades on addition of very small quantities of acid, rapidly returns again, and once more fades on further acidification. This behaviour negatives the presence of anything more than very small amounts of hydroxy-sulphide (or what is the same thing, monosulphide), since hydroxy-sulphide titrates to hydrosulphide before the end-point to phenol phthalein is reached. It is therefore highly probable that the initial alkalinity to phenol phthalein is simply due to slight hydrolysis of the higher polysulphides. A definite end-point could not be determined owing to progressive dissociation. The re-solution of the sulphur first precipitated by acid or iodine was noted with this dip, just as in the case of the caustic soda and sulphur preparation. The amount of sulphate and sulphite is also very small, so that for practical purposes we may regard the dip as consisting of calcium pentasulphide and calcium thiosulphate.

⁶ Ramsay : *Journal Agric. Science*, Vol. VI, pt. 4, December, 1914.

According to the equation



we should have one-third of the lime and one-sixth of the sulphur passing into thiosulphate. As will be seen from the analysis however, the amount of thiosulphate present in the dip is considerably higher, so that if the equation represents the main reaction there must be some secondary reaction resulting in the further formation of thiosulphate—either an atmospheric oxidation or a further reaction in which hydrolytic sulphuretted hydrogen is expelled by boiling, so allowing of further union of the corresponding amount of lime with free sulphur, in accordance with the main reaction. That this is probable is indicated by the fact that on boiling the dip for any length of time, sulphur is slowly deposited with concomitant formation of thiosulphate or sulphite.

According to the primary equation 168 parts of lime combine with 384 parts of sulphur—an approximate ratio of 1:2.29. In actual practice the combining ratio in dip-making is somewhat lower, as a result of the secondary increase in the amount of thiosulphate. In the analysis already given it is 1:2.11. The proportions of 15 lb. unslaked lime to 25 lb. of sulphur, or 1:1.67, recommended in the directions for dip-making, do not, therefore, represent the most favourable combining ratio.

From the analysis already given, only 0.934 per cent. of lime appears in the dip. According to the method of dilution 1.5 per cent. should appear if all the lime used had been taken up—or rather less, considering the amount of concentrated fluid left behind in the barrel along with the sediment. The lime which had been used was therefore subjected to analysis, and although purchased as burnt lime was found to be largely slaked and partially carbonated. Although almost completely soluble in acid, and containing only a trace of soluble silica, it showed a total lime content of only 82.6 per cent., of which 6.6 per cent. was present as carbonate. Taking the free lime present as 76 per cent., the 15 lb. used only corresponds to 11.4 lb. of true lime, and a maximum of 1.14 per cent. could therefore be expected in the dip. The true proportion of the free lime which had passed into solution could not be calculated from these figures, since the precise amount of concentrated solution left behind in the sediment was not known. A small quantity of dip was therefore made up on the laboratory scale, using the same proportions of material: 15 grammes of lime, 25 grammes of sulphur, and 250 c.c. of water. After boiling for forty minutes the concentrated fluid was filtered and diluted to 1000 c.c., the sediment being washed with the diluting water. Analysis showed 1.05 lime equivalent in solution, or 70 per cent. of the commercial lime used. Considering the free lime in the sample (76 per cent.) this represents a utilization of 92 per cent. of the lime which could theoretically have passed into solution.

The ratio of theoretically available lime, $15 \times 0.76 = 11.4$ parts, to theoretically available sulphur 25 parts, is 1:2.2, or a little below that required for the equation (2.29) and a little above that (2.11) actually required to account for all the lime as thiosulphate and pentasulphide. The solution of lime was therefore not limited in this case by the amount of sulphur.

(b) *Conditions controlling the Solution of Lime and Sulphur.*

To determine, more generally, the conditions controlling the utilization of lime and of sulphur at the usual boiling bulk, three tests were carried out:—

(a) Using pure lime with excess of sulphur.

Quantities: 20 grammes calcium hydroxide (73.0 per cent. CaO), 50 grammes sulphur.

(b) Using a poor quality of commercial burnt lime with excess of sulphur. The proximate analysis of the lime used was as follows:—

	Per cent.
Total CaO	63.3
MgO	6.9
CO ₂	6.0
Soluble silica	5.6
Insoluble matter	4.5
H ₂ O, Fe ₂ O ₃ , Al ₂ O ₃ , etc.	13.7

Quantities:—

Lime: 20 grammes.

Sulphur: 50 grammes.

(c) Using pure lime in large excess of the sulphur—in the proportions required for the formation of monosulphide.

Quantities:—

100 grammes calcium hydroxide.

41 grammes sulphur.

In each case the sulphur was worked into a paste with water in a mortar, and washed into a 500 c.c. Erlenmeyer flask. The lime was added and then sufficient water to bring the total water up to 250 c.c. The mixture was boiled gently for forty minutes with frequent agitation, filtered hot and made up to 1000 c.c. with cold air-free water, the diluting water being used to wash the sediment. The following analyses represent the composition of the resulting dips, per 100 c.c.:—

	(a) Per cent.	(b) Per cent.	(c) Per cent.
Total lime	1.429	0.645	2.130
Total sulphur	3.303	1.465	4.000
Sulphur as thiosulphate	0.622	0.280	0.845
Sulphur as sulphate and sulphite	trace	trace	0.050
Total sulphide sulphur	2.681	1.185	3.105
Monosulphur equivalent	0.506	0.228	0.735
Free CaO in solution	—	—	(0.06)

For (a) and (b) the ratio of monosulphide sulphur to total sulphide sulphur is approximately 1: 5.3 and 1: 5.2, respectively. Here, again, we have a quantity of sulphur above that required for the pentasulphide. In (c) we have a ratio 1: 4.2, corresponding to a mixture of pentasulphide and tetrasulphide, while at the same time there is also an appreciable amount of free lime present. This free lime has probably been acquired from the aqueous washings of the sediment, but at the same time free lime may theoretically occur in a dip whenever the sulphur used is much below that required to form the pentasulphide. The formation of higher sulphides from lower sulphides occurs very rapidly, while the combination between sulphur and free lime in solution, or the reduction of higher polysulphides to lower, takes place slowly. So long, therefore, as *excess* of solid lime

is present, small quantities of free lime are present in solution, and the removal of this only takes place slowly in the hot medium after sedimentation of residual lime. The precise amount of free lime finally in solution may therefore vary from a mere trace up to that quantity soluble in the volume of boiling concentrated dip concerned. This latter, however, is itself very small, and the amount of free lime which can theoretically occur in a dip is negligible for practical purposes, however badly the dip is made.

In (b) there was also present a trace of magnesia derived from the magnesia in the lime used, but this was too faint to be of any consequence.

In (a) we have 14.29 grammes of lime dissolved out of the $(20 \times 73.0) = 14.6$ grammes used, i.e. 97.8 per cent.

Where, therefore, approximately pure lime is used with excess of sulphur, utilization of lime is almost quantitative, and the solution of sulphur is limited by the quantity of lime.

In (c) where large excess of lime was used, solution is limited by the amount of sulphur. Of the 41 grammes of sulphur used, 40 grammes, or 97.5 per cent., is found dissolved in the dip. Of the 100 grammes of slaked lime used, equivalent to 73 grammes of oxide, only 21.3 grammes CaO, or 30 per cent., is found in solution. The remainder is simply left over, uncombined with sulphur in any form. The absolute amount of lime used is thus only slightly more than in the case where excess of sulphur was used, enough being taken up to form a mixture of tetrasulphide and pentasulphide.

We regard this experiment as disposing of the contention that polysulphides lower than the tetrasulphide exist in the dip. There was here an opportunity for the formation of any derivative from the monosulphide upwards, yet not even the di- or trisulphide was formed.

In (b) the amount of lime passing into solution is 6.45 grammes, equal to 32.2 per cent. of the commercial sample used. Considering the analysis of the sample, we have silica 5.6 per cent. and carbon dioxide 6.0 per cent., distributed over magnesia 6.9 and calcium oxide 63.3 per cent. From these figures it is difficult to gauge the proportion of true calcium oxide present, since the distribution of base and acid is not known. It is sufficient, however, to note that with this commercial lime of low grade, only about half of the total calcium oxide present was actually utilized under practical conditions.

Before continuing the consideration of the quality of lime as it affects dip-making, we may briefly consider the influence of the proportion of water used in boiling the lime-sulphur mixture. From the results already given, the use of quantities of water larger than the 25-30 gallons recommended, obviously has no advantage to offer. At this boiling bulk, solution of both sulphur and lime is practically complete according to whether lime or sulphur be used in excess of the ideal ratio, $\text{CaO} : \text{S} :: 1 : 2.3$. It would, however, be of some advantage if the boiling bulk could be diminished, thus allowing of the use of smaller utensils. Dip-making was therefore tried on the laboratory scale at volumes approximately corresponding to 30, 20, and 10 gallons, as follows:—

	(a)	(b)	(c)
Ca(OH) ₂	20 grm.	20 grm.	20 grm.
Equiv. to CaO	14.6 „	14.6 „	14.6 „
Sulphur	35 „	35 „	35 „
Water	300 c.c.	200 c.c.	100 c.c.

The proportion of sulphur was purposely taken in considerable excess of that theoretically required, so as to ensure maximum solution of lime.

The following figures show the distribution and extent of solution of the lime:—

	(a)	(b)	(c)
	Per cent.	Per cent.	Per cent.
Total lime	1.400	1.380	1.060
Lime as thiosulphate	0.530	0.508	0.330
Proportion of lime dissolved	95.9	94.5	72.5
Proportion of dissolved lime appearing as thiosulphate	38	36.8	31.1

It is seen that at a boiling-bulk of over 20 gallons, approximately 95 per cent. of the lime taken appears in the dip; in this case rather less than in the previous test where nearly 98 per cent. was dissolved at a boiling-bulk of 25 gallons.

At 10-gallon bulk, solution of lime is much less perfect, and the proportion of sediment is proportionately greater. To obtain high utilization of lime, the boiling-bulk must therefore be kept high. The lower proportion of thiosulphate in (c) is also interesting. Since the calcium present in this form is below the theoretical amount expected (33.3 per cent. of the total lime), it would seem that secondary decomposition of thiosulphate occurs whenever the boiling-bulk is too low. The precise nature of this secondary decomposition was not investigated, since it is of minor importance in so far as dip-making on the homestead is concerned, and does not seem to occur at a boiling-bulk of over 20 gallons.

(c) *Quality of Lime used in Dip-making.*

Theoretically, we have several possibilities to consider. It may be (a) that the true free lime present in any commercial sample is wholly utilized, other constituents behaving merely as inert bodies; (b) that the other constituents have a depressing effect, *per se*, upon the availability of the lime; (c) that the other constituents depress the availability of the free lime simply in virtue of their mechanical action in preventing slaking to a fine powder and hence by leaving the lime in a physical condition in which it is less readily dissolved during boiling.

In regard to the purely chemical influence of constituents other than free oxide, little need be said. As the following data show, the influence of calcium carbonate and of magnesium oxide, as such, is negligible. Quantities of material were taken:—

	1.	2.	3.	4.
Ca(OH) ₂	20 grm.	20 grm.	20 grm.	—
Ca CO ₃	—	10 grm.	—	—
MgO	10 grm.	—	—	10 grm.
S	40 grm.	40 grm.	40 grm.	40 grm.
H ₂ O	250 c.c.	250 c.c.	250 c.c.	250 c.c.

The mixtures were then boiled for forty minutes with frequent agitation, filtered, diluted to 1000 c.c., and analysed:—

	1.	2.	3.	4.
	Per cent.	Per cent.	Per cent.	Per cent.
CaO	1.40	1.43	1.41	—
MgO	trace	—	—	trace
Total sulphur ...	3.06	3.15	3.12	(0.014)

In the case of magnesia alone, practically no reaction takes place on boiling with sulphur. The resulting fluid was of a very pale greenish-yellow tint, rapidly bleaching on standing. The amount either of magnesia or of sulphur, dissolved, was negligibly small. In (1) the presence of magnesia neither raised nor depressed the solution of lime or of sulphur. In (2) the presence of chalk had no effect. From the chemical point of view both substances behave as inert bodies.

The influence of combined silica, as such, would probably also be negligible, although since the precise distribution of the silicate in a sample of burnt lime is not known, this point is not so easily tested.

The fact still remains, however, that a low-grade lime does not yield nearly so much of its total oxide as would be expected from its analysis—even after making full allowance for oxide which may be in combination as carbonate and silicate. The explanation is probably to be wholly found in the mechanical effect of the foreign constituents. Magnesia, carbonate, and silicate, in virtue of their binding and protective influence upon the sample as a whole, prevent effective slaking to fine powder and so lower the rate of solution of the actual free lime present. The subsequent reaction between the sulphur and the boiling lime-water is thus retarded and an inferior dip results.

The influence of the physical factor was incidentally well illustrated in one trial in which a lime used in making up a dip for the laboratory tank was found to slake badly and only yield 60 per cent. of its total calcium oxide to sulphur. On grinding up a sample of this lime in a mortar and repeating the dip-making on the laboratory scale, 82 per cent. of the total calcium oxide passed into solution.

In general practice, then, it is highly important that the lime used be of high grade. Although, as will be shown later, a dip much below the concentration obtained from a high quality lime is still effective in curing scab, the use of low quality lime is uneconomical and liable to give uncertain results.

The lime-sulphur dip has been strongly recommended by the Division of Sheep, but its use has been considerably restricted by the difficulty of procuring reliable lime. In order to ascertain the quality of lime generally available for dip-making, a number of sheep inspectors throughout the Union were circularized and twenty-five random samples so procured. A statement of the origin of each lime was asked for, but no information other than the locality in which the material was offered for sale could be obtained. Many of the samples were forwarded simply as "lime" without further specification. Some were stated to have been sold as burnt lime and others as slaked lime. No attempt has therefore been made to classify the samples according to their geological origin.

A good few of the samples were already in a fine state of division, but others although fully slaked, as a thermal test showed, were very lumpy. Under these conditions it was not possible to determine exactly the value of each lime for dip-making, since different users would vary in the extent to which they took the trouble to break down the lumps. There was not one really first class "fat lime" in the whole batch. In the case of those few which were still partially unslaked, further slaking took place very imperfectly and did not have the effect of reducing the material to the desirable finely divided condition.

A practical test was carried out on each sample by preparing a dip on the laboratory scale, using large excess of sulphur and estimating the total calcium oxide passing into solution. Owing to exigencies of sampling, the limes were first ground to a medium powder by passing once through a coffee mill, and the results therefore show up the samples as better than they really are. The data given in the following table (III) are more or less "optimum figures," since it is quite certain that most of the samples would be used in practice in a less suitable mechanical condition. In the last column the "dipping efficiency" is expressed as the percentage of sample appearing in the dip as dissolved lime. Total calcium oxide, carbon dioxide, and magnesia, are also given—magnesia being only estimated when it exceeded 1 per cent., is indicated by a blank when it fell below this figure.

The source of the sample is indicated by the name of the sheep inspector who procured it and the district in which it was for sale.

Table III.

Source.	Total CaO.	MgO.	CO ₂ .	Dipping efficiency.
	%	%	%	%
Mr. Blignaut, Maritzburg ... 1	63.8	25.7	4.5	53
" ... 2	59.8	22.1	5.3	48
" ... 3	53.2	21.4	6.1	40
Mr. Steenkamp, Garies ... 1	62.1	—	13.4	38
Given River ... 2	63.1	—	13.7	40
Strandfontein ... 3	60.0	—	9.2	41
Steenkopf ... 4	54.5	—	7.7	37
Mr. Devenish, Prieska ... 1	46.5	1.6	7.8	19
" ... 2	49.5	2.2	15.0	12
Mr. Kruger, Dordrecht ...	65.0	—	10.6	38
Mr. Van Heerden, Lime Banks ... 1	43.3	5.4	8.9	19
" ... 2	48.3	6.2	9.0	22
Mr. Keightly, River View ... 1	63.2	4.1	8.4	30
" ... 2	60.2	5.7	2.2	40
Mr. Rosseau, Upington ...	60.0	—	4.2	50
Mr. Marais, Pietersburg ...	58.1	—	7.0	37
Mr. Oosthuizen, Krugersdorp ...	78.6	—	3.9	70
Mr. Veater, Vryburg ... 1	54.2	—	13.2	25
" ... 2	65.2	—	5.7	36
Mr. Murray, Vrede ...	42.0	23.1	10.1	11
Pretoria Merchant ...	81.6	—	5.2	71
Unknown Sample ... 1	39.9	3.2	7.5	15
" ... 2	46.2	3.4	12.7	16
" ... 3	70.2	—	3.5	59
" ... 4	40.2	17.1	9.4	14
Laboratory Prepared Lime ...	95.9	—	1.0	94
" " Slaked Lime ...	72.0	—	0.7	70

It will be noted from the figures in the last column, which represent the actual percentage of calcium oxide extractable from the sample by boiling with sulphur, that the samples on the whole are very poor. Some of them, notably "Vrede," "Unknown Sample 4," and "Prieska 2," are so bad that, even regarded as slaked limes, they yield dips less than one-fifth as concentrated as dips prepared from first-class slaked limes would be. Even the best sample (Krugersdorp) is indifferent if regarded as a "burnt lime" and used for dip-making on the 15-lb. basis. Regarded as a slaked lime, as it largely was, it is excellent. The samples from Garies,

Given River, and Strandfontein were all sold as "shell lime," and regarded as such are therefore of less than half the value of a really good "burnt shell." As a matter of fact, however, all three samples were found to be almost wholly slaked when tested by the thermal reaction and, as the data in the third column show, contained a large proportion of carbonate. These samples contained very little insoluble matter or soluble silica, and the limestone from which they were originally derived must have been of good quality. The relatively poor quality of the three limes as such is either attributable to imperfect burning, or to reversion by atmospheric slaking in storage. The somewhat low solubility of the free lime actually present, as calculated by deducting that present as carbonate, is doubtless due to the mechanical condition of the samples, which even after passing the coffee mill still contained some particles of gritty character.

The two samples from River View were both sold as unslaked. Sample 1 would therefore only give a dip of less than one-third, and Sample 2 of less than one-half, of the expected strength.

Most of the samples which yielded very little lime on boiling with sulphur, notably "Vrede," the two Prieska limes, and "Unknown 4," contained relatively large amounts of "soluble silica" and of insoluble matter. It was not considered worth while making complete analyses of all these limes, since for the purpose in view the general estimation of solubility to sulphur was regarded as sufficient.

Taking the whole batch of samples as representing limes available for dip-making in various parts of the Union, the position of the purchaser must be regarded as very unsatisfactory.

In most cases lime is bought already slaked, and this is in itself to be regarded as a disadvantage, since rough judgment of the sample by its slaking quality is no longer possible. It is evident that the strength of home-made lime-sulphur dips will vary enormously—the worst sample giving only about one-sixth of the anticipated concentration.

It must, of course, be pointed out that the limes under consideration are not all regularly used for dip-making. The sheep inspector or stock owner uses his own discretion, and in most cases doubtless rejects really hopeless material and abandons the lime-sulphur preparation in favour of another dip. The dark coloured Vrede sample, for instance, was so obviously unsuitable that it would probably be rejected at sight. In those cases, however, where the lime appears to be good enough and yet only gives a dip of, say, one-half or one-third of anticipated strength, the saving grace of the dipping lies in the fact that preparations of concentration far below that given by a good lime are still effective in curing scab.

Even a lime of which only one-third passes into the dip still gives a calcium oxide equivalent of $\frac{15 \times 88.8}{1000} = 0.5$ per cent., or 0.31 per cent. in polysulphide form. This corresponds to approximately 0.9 per cent. of polysulphide sulphur, which, as will be shown later, is still quite effective in curing scab. At the same time the use of low quality lime is uneconomical and involves great waste of sulphur. The element of uncertainty is also introduced and the margin of safety is removed.

In connection with this question of the quality of lime generally available for dip-making it should be noted that, although the ratio 15 lb. of burnt lime to 25 lb. of sulphur recommended by the Sheep Division is, as already remarked, not the most advantageous one

where both ingredients are of high grade, it still offers excess of sulphur for reaction with all the commercial limes considered above. The combining ratio as found by analysis of good dips may be taken as about 1 CaO:2.1 S. Allowing a small marginal excess of sulphur, it should be made about 1:2.3 in practice. In Table III the best sample of lime shows a dip-making efficiency of 71 per cent. This was sold as a burnt lime, and hence the 15 lb. to be taken only corresponds to 10.65 lb. of available free lime, which balanced against 25 lb. of sulphur gives a ratio of 1:2.35.

(d) *Quality of Sulphur Used.*

Very little need be said on this point, since all that has been said in discussing the caustic soda and sulphur dip applies, *pari passu*, to the lime-sulphur preparation. Comparative tests have shown that the kind of sulphur is of no consequence, provided that it is pure and is not too coarse. Flowers of sulphur and ground rock sulphur are equally serviceable provided the latter be milled to the degree of fineness of the former.

(e) *Commercial Lime-sulphur Concentrates.*

So far as we are aware there is only one concentrated product on the South African market at present. This is manufactured by the Cape Explosives Co., and is marketed under the name *Capex* for use either as a sheep dip or as a spray for fruit trees. The analysis of the concentrated fluid in the drum used in the laboratory dipping trials was as follows:—

Capex.

(Percentage figures by volume, i.e. grammes per 100 c.c.)

Calcium stated as CaO	13.7
Total sulphur	37.3
Sulphur as polysulphide	36.1
Sulphur as thiosulphate	0.5
Sulphur in other forms	0.7
Specific gravity at 25° C.	1.319

(Free lime, *nil.*)

From these figures it is seen that this preparation is an excellent one and contains practically 97 per cent. of its sulphur in polysulphide form—calcium pentasulphide. The amount of thiosulphate is extremely low owing to the method of concentration—thiosulphate, being unstable in hot strong solution, is limited probably by the solubility of calcium sulphite at the temperature adopted for concentration. The absence of thiosulphate is, however, an advantage, since it is the proportion of polysulphide sulphur which determines the efficacy of the dip. This concentrate may be brought to any desired tank strength by simple dilution with water. At the request of the makers two dilutions were tested in the dipping trials presently to be discussed—1 to 18 and 1 to 40. The tank strength now recommended by the makers is 1 of *Capex* to 40 of water.

We are not concerned at present with the economics of the question, but may mention that at the time of trial *Capex* was quoted at 1s. 8d. per gallon at the factory. At the dilution recommended this preparation would cost the farmer 4s. 2d., plus cost of transport, per 100 gallons of tank dip.

The question of effective strength will be brought up again later.

The Modderfontein Explosives Co. also forwarded a sample of a lime-sulphur preparation. This concentrate is not yet on the market however, and since the makers propose to put out a product of higher grade than that supplied for trial, no detailed reference to it need be made.

3. LOOGAS AND SULPHUR DIP.

“Loogas,” a strongly alkaline plant ash, is sometimes used as a substitute for caustic soda in preparing home-made dip. The name loogas is apparently used in a comparatively wide sense, being applied to the ash obtained by the burning of particular shrubs in various localities without specific reference to a single botanical species. In the nature of things loogas would be expected to vary considerably in composition, with the mixed carbonates of sodium and potassium as chief water-soluble constituents. An attempt was made to obtain a number of samples from different areas for comparison, but the product seems to be relatively seldom used and only one representative sample was actually obtained.

This sample, sent in by Senior Sheep Inspector Wilson, of Ceres, and used in the dipping trials, showed a proximate analysis:—

	Per cent.
Moisture	18.14
Insoluble matter	29.74
Water-soluble fraction ...	52.22
of which Na_2O ...	19.82
K_2O ...	7.56

The bulk of the water-soluble base was present as carbonate, with minor amount combined as soluble silicate, phosphate, chloride, and sulphate. The active constituents from a dip-making point of view are the carbonates of sodium and potassium, and probably also the easily dissociated soluble silicate. “Loogas and sulphur” dip is prepared in practice by mixing 25 lb. of well-sifted sulphur with 25 lb. of powdered loogas into a paste with water, throwing into a pot containing 25 to 30 gallons of boiling water, boiling for twenty-five minutes, and then sedimenting off as in the case of the lime-sulphur dip.

A priori very little reaction could be expected to take place between sulphur and sodium carbonate even in boiling solution, and the dip would therefore be expected to contain very little dissolved sulphur. That this is actually the case is indicated by the following proximate analysis of the loogas-sulphur dip as used in the dipping trials:—

	Per cent.
Total Na_2O	0.495
Total K_2O	0.189
Total sulphur	0.193
Total sulphide sulphur ...	0.139
Thiosulphate sulphur	0.047

In addition, small amounts of preformed sulphate, phosphate, and chloride were present.

The dip was of a pale yellow colour, strongly alkaline to phenol phthalein owing to the large excess of residual sodium and potassium carbonate. As will be seen from the analysis, only a very small proportion of the base is in combination with sulphur. Owing to the large amount of insoluble matter in the loogas, the sediment in the

settling barrel was very large and contained practically all the sulphur unacted upon. Since this sediment, containing about 23 lb. out of the 25 lb. of sulphur used, is thrown away, the absurd waste involved in the use of the loogas dip is apparent.

Taking the total sulphide sulphur as the active constituent, it is seen that the amount present is less than a quarter of that normally present in a home-made caustic soda and sulphur dip. *A priori*, therefore, one would not expect the loogas dip to be at all reliable for the cure of scab.

4. EFFICACY OF THE SULPHUR DIPS.

The actual supervision of the dipping of the sheep, and the examination for cure, were carried out by Mr. Bedford, to whose report reference should be made.

The following Table IV indicates the results obtained with correctly prepared dips. All the sheep used had been artificially infected with scab, and at the time of dipping were in a much worse state than is common even amongst the most heavily infected members of a naturally infected flock. The sheep were examined before each dipping, again a week later, and thereafter at intervals during the six months following—in order to make quite sure that cure had been complete. The dipping interval was ten days and the immersion period two minutes.

A dipping trial with a 2.5 per cent. solution of sodium thiosulphate (hypo. crystals), i.e. of sodium-equivalent much above that of the soda-sulphur dip, and of sulphur content not much below, was made in order to determine the value of this constituent of the sulphur dips.

Table IV.

DIP.	CAUSTIC SODA AND SULPHUR.		LIME AND SULPHUR.			Loogas and Sulphur.	Sodium Thiosulphate.
	Home- made.	O'Gor- man's.	Home- made.	Capex, 1 : 18.	Capex, 1 : 40.		
Per cent. sulphur as polysulphide (i.e. "total sulphide sulphur")	0.644	0.313	1.517	1.720	0.881	0.139	—
Per cent. sulphur as thiosulphate	0.143	0.224	0.430	0.026	0.012	0.047	0.645
Number of sheep dipped—							
(a) once	6	6	6	—	—	—	—
(b) twice	6	6	6	6	6	6	6
Number of sheep upon which acari could be found a week after—							
(a) first dipping	none	3	1	none	none	2	all
(b) second dipping	none	1	none	none	none	none	all
Number of sheep upon which acari could be found up to six months after—							
(a) first dipping	1	3	3	—	—	—	all
(b) second dipping	none	1	none	none	none	none	all

It will be seen that complete cure was effected with the three home-made dips after dipping twice within the life-cycle of the scab parasite. A single dipping, however, was not found effective. In several cases eggs, probably protected by crusts, hatched out after the first dipping, but were caught as acari on the second dipping.

The test with thiosulphate was completely negative, and the dipping had little, if any, effect upon the scab parasite. It is therefore fairly safe to assume that the value of thiosulphate sulphur is negligibly small, and that the efficacy of a sulphur dip is to be measured by its content in the dominant remaining constituent—polysulphide.

In the case of O'Gorman's concentrated dip, one sheep escaped cure, even after the second dipping, a fact probably attributable to the low proportion of polysulphide sulphur at the dilution recommended by the makers. If a dilution of 1:15 instead of 1:30 were used, the amount of polysulphide sulphur at tank strength would then correspond to that of the home-made dip, and reliable results might then be expected.

The results with the loogas and sulphur dip are interesting and unexpected. As already remarked, good results could not be expected from so low a proportion of polysulphide sulphur. Yet after two dippings within the usual interval, cure was apparently complete and no acari were found six months later. The result is not easily explained. A low concentration of total sulphide sulphur may be effective in some cases, and the success in this instance, where only six sheep were dipped, is perhaps attributable to chance. There is no reason for assigning any virtue to the large proportion of sodium and potassium carbonate present in the loogas dip, and the failure of O'Gorman's, of polysulphide content twice as high as that of the loogas and sulphur preparation, at least suggests that the latter is not to be relied upon in general practice. Since the loogas dip is not much used, no repetition of the trial was included in Mr. Bedford's tests, but the point may be taken up again later in connection with a study of the mode of destruction of acari by sulphur preparations.

In regard to the efficacy of the lime-sulphur dip, it should again be noted that according to the proportions used for the home-made fluid the lime is in excess of that theoretically required for combination with the sulphur, and that the whole of the 25 lb. of sulphur used could therefore pass into solution. On the basis of dilution to 100 gallons the total sulphur content of the dip could rise to 2.5 per cent. Assuming that in presence of excess of lime the tetrasulphide is formed rather than the pentasulphide (*vide* earlier) the total sulphide sulphur would range a little below 2 per cent. Even with an indifferent quality of lime it would range over 1.5 per cent., as in the case recorded in Table IV. The amount of polysulphide sulphur to be expected in a home-made lime-sulphur preparation is therefore from two to three times as high as that present in the home-made caustic soda and sulphur dip.

The latter dip, on the other hand, contains a large excess of free sulphur, and it is difficult to know whether this comparatively coarse (often caked) material has any real value or not. As already mentioned, it is carried off, irregularly distributed in the fleeces of the sheep, and unevenly apportioned according to the order in which the sheep enter the tank—those dipped first receiving most and those dipped last receiving least. In distinction to this the polysulphide sulphur, being in solution, impregnates the fleeces very uniformly, and, as a result of rapid subsequent decomposition on exposure to the air, sulphur is deposited in a very fine state of division upon the skin and throughout the wool of the sheep. It is therefore highly probable that the excess of sulphur in suspension in the dipping fluid

has a negligible effect when compared with the dissolved sulphur, and it is probably accurate to take the "total sulphide sulphur" of a dip as the measure of its efficacy. In this case it is apparent that the home-made lime-sulphur preparation contains an unnecessarily high proportion of dissolved sulphur. By analogy with the soda-sulphur dip, a lime-sulphur fluid containing about 0.7 per cent. of total sulphide sulphur should be quite effective in curing scab. It is therefore interesting to note that the test with Capex at a dilution corresponding to 0.88 per cent., or about half that of a first-class home-made dip, was found to be thoroughly effective in Mr. Bedford's tests. Detailed investigation of the *minimum concentration* of polysulphide sulphur at which cure of scab can be wholly relied upon, did not come within the scope of the present scheme of work, but it may possibly be taken up later. The settling of the point would, however, involve a comparatively elaborate series of dipping trials, since with low concentrations the element of chance is magnified and would have to be eliminated by multiple tests. It was considered advisable to first give the sheep inspectors of the country an opportunity of reporting their practical experience with Capex 1:40, before proceeding to experiment with higher dilutions.

From Table IV the effective concentration is suggested as lying between 0.31 per cent. and 0.64 per cent., and so far as the caustic soda and sulphur dip is concerned it would therefore be unwise to consider any reduction in the concentration of the home-made preparation. In regard to the home-made lime and sulphur dip, dilution to tank strength by addition of 100 gallons of water, instead of "making up" to 100 gallons (by the addition of about 70 gallons of water) is certainly advisable, and if a good quality of lime were *always* used the dilution could be carried much further. The objection to the actual recommendation of higher dilutions, lies in the uncertainty as to the quality of lime being used. In dealing with commercial lime-sulphur concentrates of guaranteed composition this objection obviously disappears.

In two further experiments to which reference will be made later on, batches of sheep were dipped in 0.5 per cent. caustic soda solution, and in saturated lime-water. In neither case was an effective cure established, and it is therefore safe to conclude that any residual free caustic soda in a badly made soda-sulphur dip, or any trace of free lime which may possibly occur in a lime-sulphur dip, does not contribute towards the parasiticial properties of the dip concerned.

5. PROBABLE DECOMPOSITION OF THE SULPHUR DIPS.

The mode of decomposition of the sulphur dips on exposure to the atmosphere in the fleeces of dipped sheep is of some interest. From the practical point of view it is important to know whether any substances of a depilatory nature are formed in the course of decomposition. As will be shown presently, the higher polysulphides of sodium and calcium are not depilatory in dilute solution. The hydrosulphides and monosulphides are, on the other hand, depilatory, and even in extremely dilute solution have a damaging effect on wool.

(a) *Caustic Soda and Sulphur Dip*.—To follow the general course of atmospheric decomposition in aqueous solution, 10 c.c. quantities

of this dip were exposed to air in thin layers in a series of 300 c.c. Erlenmeyer flasks, and tested at various intervals.

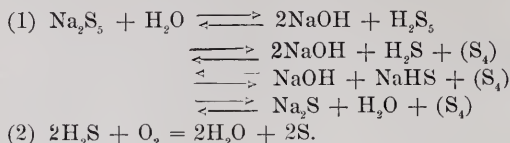
The original *immediate* alkalinity to phenol phthalein (evanescent) was about 0.2 c.c. $N/_{10}$. In the course of a day sulphur slowly deposited with gradual decrease in the amount of polysulphide, and gradual increase in the amount of thiosulphate, but with little or no alteration in alkalinity towards phenol phthalein. In from sixteen to twenty hours, during which time a small quantity of water had evaporated, the sulphide reaction to nickel sulphate was found to disappear altogether, and analysis of the colourless residual fluid showed the bulk of the base originally present as polysulphide to be now present as thiosulphate. Different flasks were found to vary slightly, probably owing to slight differences in the shape of the bottom and consequent differences in the depth of layer exposed to atmospheric oxidation. The following data, however, represent the general course of decomposition:—

10 c.c. Dip.				EXPOSURE.			
				Fresh.	3 Hours.	7 Hours.	20 Hours.
$N/_{10}$ acid to phenol phthalein	0.2 c.c.	0.1 c.c.	0.2 c.c.	0.2 c.c.
" methyl orange	8.1	5.8	3.9	0.8
$N/_{10}$ AmZnCl ₂ to nickel sulphate	7.8	5.6	3.5	nil
$N/_{10}$ I ₂ on Zn filtrate, for thiosulphate	2.2	3.3	4.4	5.9
Sulphate and sulphite by BaCl ₂	trace	trace	trace	trace

Since 1 c.c. $N/_{10}$ I₂ for thiosulphate corresponds to 2 c.c. $N/_{10}$ acid or am. ZnCl₂ for titratable base, we have initially a total base equivalent of $8.1 + (2.2 \times 2) = 12.5$ c.c., corresponding to 0.5 per cent. NaOH equivalent. Finally, after twenty hours' exposure and disappearance of the sulphide reaction, we have $5.9 \times 2 = 11.8$ c.c. of the total base present as thiosulphate and 0.8 c.c. present as carbonate and bicarbonate. We may take the difference of 0.3 c.c. between the "monosulphur equivalent" (to am. ZnCl₂) and the total acid titration as representing the initial amount of carbonate present. We have, therefore, a *slight* formation of carbonate, but a main formation of thiosulphate. Formation of neither sulphate nor sulphite could be detected, and the transformation of polysulphide to thiosulphate is therefore almost *quantitative*.

At no stage of decomposition does the alkalinity towards phenol phthalein rise, and therefore at no time does any appreciable amount of monosulphide exist in solution. At no time does the zinc titration to nickel rise above the acid titration to methyl orange, and therefore at no time does any *appreciable* quantity of hydrosulphide exist free in solution. This, of course, is only to be expected, because, since hydrosulphide and monosulphide readily take up free sulphur in the cold, even in exceedingly dilute solution, to form higher polysulphides, and since sulphur is slowly deposited by atmospheric oxidation, it is almost inconceivable that monosulphide could be an intermediate decomposition product of pentasulphide.

As to the *modus operandi* of oxidation, it seems probable that the true oxidation concerns only the traces of hydrolytic sulphuretted hydrogen :—



At any given moment a trace of sulphuretted hydrogen may be supposed to be present, owing to slight hydrolysis of pentasulphide. This may be supposed to oxidize to water and free sulphur in the usual way, at the same time bringing about separation of the hydrolytic equivalent (four atoms) of polysulphide sulphur. The equivalent amount of liberated caustic soda can then be regarded as reacting with the separating sulphur in accordance with the major reaction mentioned earlier :—



Thiosulphate is thus formed with concomitant reformation of pentasulphide, which in turn undergoes the same oxidative change. Finally all the base originally present appears as thiosulphate, while the excess of sulphur over that required for formation of this compound is visibly deposited as free sulphur.

It is supposed that the caustic soda which is liberated as sulphuretted hydrogen oxidizes, reacts not with the sulphur after deposition but rather with *nascent* sulphur on the verge of separation. Although caustic soda does not react appreciably with free sulphur in the cold, the reaction proceeds fairly rapidly at 35° to 40° C. (section 1), and probably therefore very readily in the cold when the sulphur is offered in nascent form.

This type of oxidation appears highly probable, since the reaction of the oxidizing medium remains neutral to phenol phthalein throughout. If, on the other hand, the hydrosulphide or monosulphide, present in hydrolytic traces, suffered oxidation as such, with deposition of sulphur in excess of the monosulphide, the reaction should become progressively alkaline to phenol phthalein as oxidation proceeds. That oxidation does not concern hydrolytic monosulphide as such is also suggested by the fact that a solution of monosulphide of strength equivalent to that of the dip (approximately N/10) only undergoes atmospheric oxidation very slowly. Whereas under the shallow layer conditions already indicated, a polysulphide solution is completely oxidized within one day, monosulphide still gives a marked nickel reaction after five days.

In support of the type of oxidation just outlined, in which the thiosulphate formation arises by direct action between hydroxide and sulphur, is the fact that if caustic soda be added to the solution of polysulphide in amount sufficient to account for all the sulphur, no deposition of sulphur takes place and a corresponding amount of hydroxide is converted into thiosulphate.

In testing this point 20 c.c. quantities of N/10 NaOH were added to 10 c.c. of dip in 300 c.c. Erlenmeyer flasks and exposed to air, control flasks being taken with 20 c.c. of water instead of caustic soda, so as to compare rates of oxidation in the same depth of layer.

of sulphur available is in considerable excess of that required to form thiosulphate, and part, therefore, always in process of deposition, the disappearance of the yellow polysulphide colour is practically simultaneous with the disappearance of the sulphide reaction to nickel and no marked formation of monosulphide can actually be recognized. Monosulphide cannot exist free so long as sulphur is being deposited.

On titrating a simple polysulphide solution after complete atmospheric decolorization, it was noticed that the small residual amount of base titratable to acid did not correspond wholly to carbonate, the titration to methyl orange (0.8 c.c.) being more than double that to phenol phthalein (0.2 c.c.). Since the presence of hydrosulphide was excluded by the nickel reaction, the presence of bicarbonate is suggested. The amount, of course, is very small, but there is still the suggestion that a little carbonate was formed during exposure to air and that part of this carbonate subsequently passed over into thiosulphate with concomitant reliberation of carbon dioxide to form bicarbonate with the residue. At any rate it was considered of interest to determine whether sodium carbonate could act like sodium hydroxide in disposing of polysulphide sulphur as thiosulphate. *A priori* this seemed likely, since carbonate is capable of slowly dissolving free sulphur when the two are boiled together for any length of time.

To test the point, 10 c.c. quantities of dip, with addition of 20 c.c. $N/_{10}$ Na_2CO_3 , were exposed in Erlenmeyer flasks as before. It was noted that although a certain amount of sulphur was slowly deposited in spite of the excess of carbonate, *part* of the carbonate was converted into thiosulphate. The following titration values indicate the behaviour of the fluid, initially, and after the disappearance of the nickel reaction:—

		Fresh.	Decomposed.
$N/_{10}$ acid to phenol phthalein (evanescent)	...	10.3 c.c.	6.9 c.c.
„ methyl orange (final)	28.1	17.2
$N/_{10}$ $AmZnCl_2$ to nickel sulphate	7.9	nil
$N/_{10}$ I_2 for thiosulphate	2.2	7.7
Sulphate and sulphite to $BaCl_2$	trace	trace

The control with water in place of $N/_{10}$ Na_2CO_3 gave 5.9 $N/_{10}$ I_2 for thiosulphate, corresponding to 11.8 c.c. $N/_{10}$ base. The 7.7 c.c. $N/_{10}$ I_2 for thiosulphate formed in presence of sodium carbonate corresponds to 15.4 c.c. of base, and hence $15.4 - 11.8 = 3.6$ c.c. of base, originally present as carbonate, has been converted into thiosulphate. From the acid titration values we have $17.2 - (6.9 \times 2) = 3.4$ c.c. of residual sodium carbonate converted into bicarbonate. Reaction between carbonate and nascent sulphur rendered available by oxidation, therefore takes place. The figure 7.7 c.c. $N/_{10}$ I_2 as compared with 9.4 c.c. $N/_{10}$ I_2 in the previous case where caustic soda was used in place of sodium carbonate, together with the fact that deposition of sulphur occurs in presence of carbonate but not in presence of hydroxide, indicates the carbonate as much less active in taking up the sulphur offered. Apparently the oxidation of hydrolytic sulphuretted hydrogen (in thin layers of liquid) is more rapid than the reaction between carbonate and separating sulphur.

In passing it may be mentioned that dilute ammonium hydroxide behaves in much the same way as sodium carbonate. It, too, serves as a base for thiosulphate formation with nascent sulphur, although, as already known, the reaction between strong ammonia and *free* sulphur is very slight, even at the boiling point.

Returning to the oxidation of polysulphide solutions in absence of free, or potentially free, base, we have still to consider the decomposition taking place in the fleeces of the sheep when a very large surface is exposed both for evaporation and for oxidation, vastly larger than in the "shallow layer" oxidation already considered, where conversion of polysulphide to thiosulphate has been shown to be almost quantitative. If, in the wool itself, oxidation proceeds faster than evaporation, we should expect thiosulphate formation to again predominate without increase, at any moment, of the concentration of sulphide. If, on the other hand, evaporation proceeds faster than oxidation, we should expect an increase in the concentration of polysulphide with possibly depilatory action on the wool. To test this point, large plugs of cotton wool were placed in a series of Petri dishes and thoroughly saturated with 10 c.c. of dip. The plugs were then teased out and left exposed to air. At various intervals after exposure they were either tested direct with nickel sulphate, or washed out with distilled water for analysis. It was found that according to the tightness of packing of the cotton wool the rate of oxidation and evaporation could be controlled at will, but that oxidation was always more rapid than evaporation. If the plugs were well teased out so as to expose a very large surface to the atmosphere, and occasionally turned and retested, it was found that complete oxidation could occur well within two hours, although the wool was then still soaking wet. By washing out with water at this point, the sodium originally present as polysulphide was found to have been practically wholly converted into thiosulphate, without formation of either sulphate, sulphide, monosulphide, or more than a small proportion of carbonate. The filtered washings were practically neutral to phenol phthalein, alkaline to methyl orange, and gave no reaction to nickel sulphate, or to barium chloride in acid solution after decomposition of thiosulphate by iodine. It was difficult to determine the exact conversion of polysulphide to thiosulphate owing to the difficulty of washing the wool with workable quantities of water, but it was gauged at from 90 per cent. to 95 per cent. .

Plugs of wool left more compactly packed, and therefore less exposed to oxidation, were also washed out from time to time. Analysis by titration of the washings as in the case of shallow-layer oxidation, gave no indication of monosulphide at any time, and simply showed unchanged polysulphide, thiosulphate, and traces of carbonate, according to the extent to which decomposition had taken place.

Similar tests were repeated with washed wool, with similar results.

We may therefore assume a simple oxidation of polysulphide in the fleeces of the sheep, without intermediate formation of depilatory compounds in effective concentration. Whatever be the conditions of drying, oxidation is more rapid than evaporation and is complete while the fleeces are still damp. As will be shown in the next section, this view is substantiated by the absence of any effect upon wool fibre during drying.

(b) *Lime-Sulphur Dip*.—The decomposition of this preparation under atmospheric oxidation may be dismissed more briefly, since it was found that oxidation of calcium polysulphides proceeds in a manner strictly analogous to that of sodium polysulphides. Oxidation in thin layer in flasks, and in films upon wool, was tested in the same manner as before. The major portion (over 90 per cent.) of the base originally present as polysulphide, is converted into thiosulphate,

and the minor remainder into carbonate, which is deposited along with the excess of sulphur. Neither sulphate nor sulphite could be detected in anything more than the merest traces, and as decomposition progressed no evidence could be obtained to suggest the presence of free hydrosulphide or monosulphide.

The atmospheric oxidation of calcium pentasulphide therefore appears to proceed in the same fashion as that of the corresponding sodium salt, oxidation of hydrolytic sulphuretted hydrogen, and reaction between hydroxide and nascent sulphur so rendered available, with formation of thiosulphate and reformation of pentasulphide, and with the presence of separating sulphur always acting in the direction of preventing the formation of appreciable amounts of lower sulphides.

In the presence of added lime water the decomposition of calcium polysulphide proceeds just as did that of sodium polysulphide in presence of free caustic soda, i.e. the added hydroxide is largely utilized for the formation of further thiosulphate by reaction with nascent sulphur which would otherwise be deposited in the free state. Part of the added lime is, however, precipitated as carbonate by absorption of atmospheric carbon dioxide. Small quantities of lime water appear to be wholly converted into thiosulphate and carbonate before decomposition of polysulphide is complete, but if the proportion of hydroxide to polysulphide is above that required for the disposal of all the sulphur as thiosulphate, appreciable quantities of monosulphide (or hydrosulphide) may appear. It is possible that even with lower quantities of free hydroxide, a very small amount of free monosulphide or hydrosulphide may be formed and coexist with undecomposed polysulphide, but its analytical detection is extremely difficult in presence of free lime and the preponderating proportion of polysulphide and thiosulphate.

In thin film on wool, oxidation of lime-sulphur dip again proceeds more rapidly than evaporation, and in very loose well aerated plugs disappearance of sulphide (nickel reaction on washings) may occur within two hours, although the wool may still be wet for many hours thereafter.

The mode of decomposition of the "loogas dip" need not be specially considered, since this dip behaves as a mixture of sodium polysulphide and sodium carbonate, the oxidation of which has already been considered.

We may therefore proceed to a consideration of the effect of the sulphur dips upon the wool and skin of the sheep.

6. EFFECT UPON THE SKIN AND WOOL OF SHEEP.

With all three sulphur dips, caustic soda and sulphur, loogas-sulphur, and lime-sulphur, properly made, no harmful effect could be detected either upon the skin of dipped sheep or upon their fleeces. If the skin was initially healthy, no irritation, or so-called "burning," could be detected the day after dipping, and if the skin was initially dry and scabby no more irritation occurred than in the case of dipping in any other sheep dip under similar circumstances.

Samples of wool were taken from various sheep immediately after dipping and again a few days after dipping. The samples were then rinsed two or three times in ether to remove most of the fat and dirt, and then carefully examined macroscopically and microscopically. In respect both to general physical properties (strength and elasticity) and to microscopical structure, no damage whatever could be detected,

and the various washed samples could only be differentiated by their labels. In regard to practical points, such as spinning and dyeing quality, nothing need be said, since tests in this direction would only carry weight if carried out by an authorized dealer in woollens. At the same time the absence of any visible alteration renders it highly improbable that even an expert wool buyer could differentiate between washed wool which had previously been dipped in the sulphur dips and washed wool which had previously been dipped in any other sheep dip.

To subject wool to more stringent test in respect to possible action by solutions of polysulphides of calcium and sodium, a quantity of clean wool from an undipped sheep was taken, roughly rinsed two or three times with ether and treated with the sulphur dips under varying conditions. The washing with ether served to remove *most* of the protective suint and therefore to give a very clean white wool much more susceptible to chemical attack than wool in its natural greasy condition. Since sodium monosulphide is known to have a violent action upon wool fibre, solutions of this salt, of varying concentrations, were used for comparative purposes, and for the purpose of determining the precise structural changes to be looked for. Quantities of about 0.03 gramme of wool were steeped in 10 c.c. quantities of the fluid to be tested, contained in 50 c.c. soxhlet flasks, and a few fibres were taken out for microscopical examination at intervals up to twenty-four hours. After a day's steeping the samples were taken out, washed, dried, and examined physically.

The following represents the preliminary series tested:—

- (a) Caustic soda and sulphur dip, usual strength ($N/_{12} Na_2S_5$).
- (b) Caustic soda and sulphur dip, twenty times dipping strength.
- (c) Home-made lime-sulphur dip, usual strength (approximately $N/_{5} CaS_5$).
- (d) "Capex" lime-sulphur preparation, diluted 1 in 5.
- (e) "Capex" lime-sulphur preparation, diluted 1 in 10.
- (f) "Capex" lime-sulphur preparation, diluted 1 in 20.
- (g) "Capex" lime-sulphur preparation, diluted 1 in 40 (approximately $N/_{10} CaS_5$).
- (h) Saturated lime water, filtered.
- (i) Calcium monosulphide (lime water + H_2S , approximately $N/_{20} CaS$).
- (j) Caustic soda alone $N/_{12}$, equivalent to concentration (a).
- (k) Caustic soda alone $N/_{20}$.
- (l) Caustic soda alone $N/_{50}$.
- (m) Caustic soda alone $N/_{100}$.
- (n) Sodium monosulphide $N/_{12} Na_2S$, equivalent to (a).
- (o) Sodium monosulphide $N/_{20}$.
- (p) Sodium monosulphide $N/_{50}$.
- (q) Sodium monosulphide $N/_{100}$.
- (r) Sodium monosulphide $N/_{200}$.
- (s) Sodium monosulphide $N/_{500}$.

Taking the last first, examination of the wool showed that with sodium monosulphide the action was extraordinarily rapid and violent. Within a few minutes at concentration (n), equivalent to the polysulphide sodium of the caustic soda and sulphur dip, the fibres were visibly swollen and showed pronounced structural alteration. The change proceeded rapidly, and on the following day the

wool was partially dissolved, and reduced to a gelatinous mass in which the fibres were enormously swollen and structurally unrecognizable. With more dilute solutions the attack of the monosulphide was proportionately slow, but even at $N/_{100}$ slight swelling and structural damage could be made out under high magnification after a few hours' steeping, or a few minutes' warming. At $N/_{200}$ Na_2S , structural damage was doubtful, even after twenty-four hours' steeping, and at $N/_{500}$ was certainly undetectable. This of course may be due to the low absolute monosulphide content as well as to the low concentration.

With caustic soda of equivalent concentration the damage to the wool fibres was much less marked than with sodium sulphide, although at $N/_{12}$ $NaOH$ structural alteration was rapid and extensive. At $N/_{50}$ it was very slow, and at $N/_{100}$ was doubtful even after twenty-four hours' soaking. The comparative effects observed with hydroxide and sulphide of equivalent strength rendered it very evident that the latter is much more depilatory than the former, and that the action of monosulphide (or hydrosulphide) is specific and not due to the alkalinity as such.

With calcium hydroxide (*h*) the action was exceedingly slight, even after steeping a whole day. With calcium monosulphide or hydrosulphide structural alteration of the fibres was again very marked.

With the *dips proper*, however, no action could be definitely detected. After three hours' steeping, fibres taken from (*a*), (*c*), (*f*), and (*g*) showed no change whatever, and even after twenty-four hours' soaking, structural damage was very doubtful—being just discernible in (*f*).

With high concentrations of the dip, damage was fairly marked. In (*b*) the caustic soda and sulphur dip at twenty times the usual tank strength, structural alteration was readily detected after fifteen minutes' steeping, and after twenty-four hours the wool was badly destroyed—being comparable to about $N/_{20}$ Na_2S . With Capex at a dilution of 1 in 5, or eight times the strength recommended for dipping, the action on wool could be made out after comparatively brief steeping, although at a dilution of 1 in 10 the damage was not great even after twenty-four hours, and, as just stated, at 1 in 20 was barely discernible.

From these tests we may conclude that although the monosulphides or hydrosulphides of sodium and calcium are exceedingly destructive to wool, the pentasulphides are *not*. Their action is exceedingly small and cannot be detected in $N/_{10}$ concentration even after several hours' soaking in the cold or several minutes' heating to $60^{\circ}C$. Such action as is manifested at higher concentrations may not even be due to pentasulphide, as such, but to traces of admixed active monosulphide or hydrosulphide.

The appearance of the wools in the tests recorded, taken out after steeping for one day, washed, and dried, varied considerably. Wool from (*n*), for instance, was no longer recognizable and dried to a caked mass. Other wools showed varying degrees of "rotting," brittleness, and loss of elasticity, the physical behaviour roughly following the structural damage as manifested microscopically. Wool steeped in Capex 1 : 10 which showed distinct but small structural alteration also showed distinct loss of elasticity after washing and drying. The wools steeped in caustic soda and sulphur dip, lime-

sulphur dip, and Capex lime-sulphur at *tank concentration* appeared quite normal and were hardly distinguishable from unsteepped wool, except in so far as they were dyed yellow from prolonged soaking. The yellow stain appeared well fixed in the fibre and could not be removed by washing with soap. This, however, is of little practical consequence, since the natural wool of sheep dipped under ordinary conditions escapes the dyeing incident to prolonged steeping of ether-washed wool, and fleeces naturally dipped in the sulphur preparations appear to wash as well as any other.

It may be mentioned that the effects noted on long steeping are not so marked with natural wool from which the protective suint has not been removed, and still less marked when the wools are merely dipped in the fluids and then spread out to atmospheric influences.

A few tests were also carried out in which samples of wool were treated with hot dip for short periods. Wool was found to stand over fifteen minutes at 80° C. in either caustic soda and sulphur or lime-sulphur dip, without serious damage to the structure (although with yellow staining of the fibre). Under the same conditions, exceedingly dilute sodium monosulphide or caustic soda brought about marked alteration.

From the general nature of the steeping experiments described, it is quite certain that with properly made dips the short immersion period of two minutes during which the sheep remain in the dipping tank has absolutely no appreciable effect upon the wool. The subsequent interval during which decomposition of the adhering fluid takes place is well within twenty-four hours, and in most cases oxidation is largely completed within the first few hours of exposure after dipping—long before the fleeces are actually dry. No damage to the wool would therefore be anticipated, and, as already mentioned, no damage is actually detectable. Wools from dipped and undipped sheep appear to be indistinguishable after washing. As illustrating this, a few microphotographs⁷ of wool fibres are given in accompanying Plates 2 and 3, to which the following scheme provides the key:—

Plate 2, low magnification—

- (1) Washed wool undipped.
- (2) The same wool steeped overnight in sodium monosulphide solution of strength equivalent to one-third that of the caustic soda and sulphur dip.

Plate 3, high magnification—

- (1) Untreated wool washed with ether.
- (2) The same wool steeped overnight in the lime-sulphur dip at laboratory temperature.
- (3) The same wool steeped overnight in the caustic soda and sulphur dip.
- (4) The same wool steeped overnight in sodium monosulphide solution of sodium equivalent one-sixth that of the dip.
- (5) The same wool steeped overnight in sodium monosulphide solution of sodium equivalent one-half that of the dip.
- (6) Wool from sheep taken a few days after the second dipping in lime-sulphur dip.
- (7) Wool from sheep taken a few days after the second dipping in caustic soda and sulphur dip.

⁷ Kindly taken for me by Mr. T. Meyer of this Division.

- (8) Wool taken from sheep a few days after the second dipping in Cooper's dip.
 (9) Wool taken from sheep a few days after the second dipping in caustic soda solution of strength equivalent to that of the caustic soda and sulphur dip.

It may be mentioned that photographing of wool fibres tends to exaggerate slight differences in the structural appearance owing to the difficulty of focussing the comparatively thick fibre as it appears under high magnification.

The loss of structure brought about even by very dilute monosulphide is well shown in fig. 5, and to a less extent in fig. 4. If, on the other hand, the photographs⁸ of the other wools were mixed up, it would not be easy to place them in their proper order again by reference to any differences in significant structural appearance. No. 2 appears to be slightly attacked, but the difference, if it is there, is more noticeable in the photograph than in the actual microscopic preparation, and is in any case of no practical significance when we consider the severe nature of the test to which the wool was subjected—over sixteen hours' steeping after removal of most of the protective suint by rinsing in ether. No. 9 of plate 3 is interesting and will be discussed presently.

Although, as indicated by the preceding steeping experiments, the correctly made sulphur dips have no effect upon the wool even when every facility for attack is offered, the case is otherwise when any considerable amount of free caustic soda or free lime is present in solution. A number of steeping trials were carried out in which varying quantities of free base were added to the dips, and it was found that relatively small absolute amounts seriously affected the wool fibres on warming to about 50° C. for fifteen minutes, or on steeping overnight in the cold. Ten c.c. of caustic soda and sulphur dip to which 0.5 c.c. of N. caustic soda was added was found to have a markedly damaging effect within a comparatively short time. The structural alteration of wool so steeped for two hours was compared with the alteration brought about by steeping for the same length of time in 10 c.c. water + 0.5 c.c. N. caustic soda. The dip containing free alkali was found to be much more depilatory than the free alkali alone, and there is therefore the suggestion that an appreciable amount of monosulphide had been formed. As mentioned in section 5, monosulphide definitely is formed during the decomposition of dip in presence of free caustic soda in excess of that required to convert all the sulphur present into thiosulphate, but the behaviour of the mixture of 10 c.c. of dip + 0.5 c.c. N. NaOH (8.1 N/10 Na_2S_5 : 5 N/10 NaOH) suggests that monosulphide may be formed even when quantities of free hydroxide below that combining limit are present. It was noted, however, that if the wool were simply dipped in the same mixture, and exposed to rapid atmospheric oxidation, the damage of the wool was scarcely noticeable—the passage of free hydroxide into thiosulphate being hastened by rapid oxidation.

With quantities of 0.1 c.c.—0.2 c.c. N. NaOH per 10 c.c. of dip, giving a free alkali concentration of N/50—N/100, or about 8 per cent. to 16 per cent. of the total sodium present, no damage to the wool could be detected even after eight to twenty hours' steeping or after

⁸ The published plates are not nearly so clear as the actual photographs in respect to scale markings.

allowing soaked wool to dry slowly in the air. It would appear, therefore, that even if the quantity of free caustic soda remaining in a badly made caustic soda and sulphur dip were as high as one-tenth of the total caustic soda used in making, it would still represent no real source of danger to the fleeces of dipped sheep.

The behaviour of the lime-sulphur dip, containing added calcium hydroxide, is very similar. Even after soaking for twenty-four hours in this dip containing free lime equivalent to $N/_{50}$ — $N/_{100}$, the wool fibres appeared to have suffered no damage, although with concentrations much above this the effects were readily noticeable. This, however, is of little consequence because, owing to the low solubility of lime in hot water and to the mode of making the dip, it is almost impossible for quantities of free hydroxide as high as $N/_{50}$ to be present—even under the most adverse circumstances.

In order to determine the effect of free alkali in *large amount* under practical conditions, an actual dipping test was carried out in which six infected sheep and one healthy sheep were put through a dip made up with only 2 lb. of sulphur (instead of 20 lb.) per 5 lb. of caustic soda and 100 gallons of water. With a similar object in view a batch of sheep were dipped in caustic soda alone (5 lb. per 100 gallons) and another batch in dilute milk of lime alone—15 lb. of lime per 100 gallons of water.

All three dippings were kindly included for me in Mr. Bedford's series of dipping trials, and were carried out twice within an interval of ten days.

In no case did the dipping effect cure of scab, but at the same time the sheep suffered astonishingly little from the treatment; much less than was expected.

The dipping in lime water (containing excess of free lime in suspension) appeared to inconvenience the animals in no way whatever, although the wool was markedly affected and (after drying) showed a harsh, brittle, inelastic character. Under the microscope, however, little or no structural damage to the fibre could be made out, and the alteration in physical nature of the fleece was therefore probably due to the deposition of particles of lime, its saturation with dissolved hydroxide, and the "cementing" action of subsequent atmospheric carbonation. This view is supported by the fact that the brittleness could be largely, though not wholly, removed by repeated washing in dilute hydrochloric acid. At any rate the fleeces would, if put on to the market, have been regarded as "rotted" and probably have found no buyer. That, however, the action was not due to chemical attack of wool-fibre by calcium hydroxide as such, is indicated by the fact that wool may be soaked for a considerable time in saturated filtered lime water, and yet, if washed at once after removal, show little or no change in physical character.

The practical interest of this dipping lies in the fact that a few odd cases have been reported in which so-called "raw lime-sulphur dip" has been used in practice. In one case reported to us, this mixture was simply made by mixing the lime (15 lb.) and sulphur (25 lb.) into a thin paste with a few gallons of water, leaving to stand overnight, and then stirring up with 100 gallons of water in the dipping tank for immediate use. Since no reaction between the lime and sulphur actually takes place in the cold, any curative virtue of the mixture lies in the uncertain effect of the free sulphur entangled in the fleeces of the sheep passing through. Six sheep were actually

dipped in this "raw dip," but although cure of scab was apparently effected in some cases, the wool of the sheep was badly affected in its general physical properties. The practice of using such a "raw" mixture is to be strongly deprecated, both as deleterious to the wool and as very uncertain in curative action.

The dipping test with plain caustic soda solution showed that the skin of the dipped sheep suffered very much less than was anticipated. After the second dipping within the ten days' interval, slight "burning" was noticeable, but in practice this would occasion the farmer no anxiety. The wool, which on the basis of the laboratory tests had been expected to suffer marked damage, showed very little structural alteration except at the tips of the fleeces. Microphotograph 9, plate (3), represents a fair sample of the wool taken at random a few days after dipping, and it is seen that the fibre appears to be quite normal. The fleeces, however, were somewhat damaged at the ends, and the fibres were matted together at the points from which draining, and subsequent slow dripping off of the caustic solution, had taken place after the sheep had left the tank. The apparently negligible effect of the solution upon the main bulk of the fleeces is probably attributable primarily to the protective influence of the wool-suint, and secondarily to the presumably rapid conversion of hydroxide to carbonate by absorption of carbon dioxide from the atmosphere.

Those sheep dipped in the caustic soda and sulphur preparation, in which the caustic soda was taken in large excess over sulphur, suffered most. The skin of the dipped sheep was visibly affected, but again the irritation was not nearly so bad as was anticipated. The skin over the legs was also slightly bruised in places, more particularly over the areas exposed to friction on kneeling or lying down.

The wool was rather badly matted, but again the damage was confined chiefly to the tips, while the bulk of the fleeces suffered relatively little. The trial, however, is sufficient to show that large excess of caustic soda in the dip in presence of reduced amounts of polysulphide *can* have a distinctly injurious effect both upon the wool and upon the sheep. The effect is probably due to the formation of monosulphide during oxidation; monosulphide, which, by resisting oxidation, may possibly remain in the fleeces until they are dry and therefore be afforded ample opportunity for exercising a depilatory action. As indicated in section 1, higher sulphides rather than lower sulphides are primarily formed even when sulphur is boiled with excess of caustic soda, but at the same time the formation of monosulphide by atmospheric oxidation in presence of excess of free base (section 5) probably begins in the tank itself, and is continued in the wet fleeces.

This trial is, nevertheless, of theoretical rather than practical interest, since it appears inconceivable to us that a caustic soda and sulphur dip could in practice ever approach the composition of this deliberately prepared mixture of heavily preponderating free caustic content. The small amounts of hydroxide, which may possibly occur in a carelessly made dip, are of much less consequence, and with ordinary care in making no free caustic alkali should be present at all.

A general summary of the major points concerning the sulphur dip may now be given.

7. SUMMARY.

1. The caustic soda and sulphur dip, as prepared according to the recommendations of the Division of Sheep, consists of a mixture

of sodium pentasulphide and sodium thiosulphate with a distribution of sulphur about four-fifths of the former and one-fifth of the latter. No monosulphide can be detected and only traces of sulphate are present. There is even the suggestion that polysulphides higher than the pentasulphide exist for a short time in the freshly prepared dip. The proportions of caustic soda and sulphur actually going into combination are approximately five to eight, and the recommended formula, 5 : 20 : $2\frac{1}{2}$ —100, provides so large an excess of free sulphur that complete utilization of caustic soda is ensured even under somewhat careless conditions of dip-making. The presence of free caustic soda in the dip need not therefore be feared if the instructions for dip-making are carefully carried out. The preliminary mixing of the sulphur to an homogeneous cream with water is the most important step to be observed, since it is upon the intimacy of subsequent contact of the sulphur with the caustic soda solution that the reaction mainly depends. Combination takes place at comparatively low temperature, and may be completed in forty minutes at 50° C., although reaction is of course more rapid at higher temperatures. If hot water is used in the preliminary mixing, the heat evolved as the caustic soda is sprinkled in suffices to keep the mixture near the boiling point, and boiling by the application of external heat is therefore unnecessary. Boiling, however, may offer slight advantages in completing the reaction in cases where the preliminary process has been imperfectly carried out. With ordinary care a dip of correct composition always results, without boiling. If the sulphur is not properly wetted in the preliminary mixing to a cream, the bulk of the sulphur may float on the surface and cake as the caustic soda is sprinkled in, and so partially escape combination. The dip may then contain residual free alkali, but not, as might be expected, any appreciable amount of sodium monosulphide.

2. The lime-sulphur dip is analogous in composition to that of the caustic soda and sulphur dip, and consists of a mixture of calcium pentasulphide with calcium thiosulphate. In preparing the dip the ingredients must be boiled, and the so-called "raw lime-sulphur dip" consists merely of a mixture of lime and sulphur. At least two parts of sulphur to one part of unslaked lime should be used whenever a really good sample of lime is available. If other proportions are used, the material present in smaller amount determines the solution of the other, higher polysulphide rather than lower being formed in all cases. If the lime is in excess, small quantities of free hydroxide may be present in the dip, but the amount is limited by the low solubility of the lime itself. Calcium pentasulphide and calcium hydroxide can, however, co-exist in the same solution even at the boiling point.

3. The loogas-sulphur dip consists mainly of a mixture of carbonates, polysulphides, and thiosulphates, of sodium and potassium, the amount of polysulphide being very low and the amount of carbonate relatively high. The reaction between alkaline carbonates and free sulphur is very imperfect even after prolonged boiling, and most of the sulphur used in making the loogas dip is therefore wasted by passing into the unused sediment.

4. The kind of sulphur used in dip-making is of no consequence provided it is finely divided and fairly pure. Flowers of sulphur and ground rock sulphur are equally suitable, but in the latter case a

guarantee of fineness of grinding should be demanded. 65° Chancel is suggested as a reasonable specification.

5. The quality of lime used is obviously of paramount importance in making the lime-sulphur dip, but limes generally available in the Union appear to be of very inferior grade. The saving grace in cases where bad limes have been used in practice, lies in the fact that concentrations of polysulphide much below that obtained in a well-made dip are still effective in curing scab.

6. The lime-sulphur and caustic soda and sulphur dips are reliable for the cure of scab. The loggas-sulphur dip also cured scab in the experimental trial carried out, but its composition suggests that its efficacy is largely a matter of chance, and that it is therefore not to be relied upon.

7. The active constituent of the sulphur dips appears to be the polysulphide, since thiosulphate, the only other important constituent, is itself ineffective. Free base, if accidentally present, does not contribute to the parasiticial efficacy of the dips. A concentration of 0.6 per cent. sulphur in polysulphide form is probably always high enough to effect cure. 0.3 per cent. showed itself as uncertain in action. The polysulphide content of the home-made lime-sulphur dip is much higher than that of the caustic soda and sulphur dip, but it is not advisable to dilute the home-made dip further than is already customary unless the lime used in making is known to be of very high quality. If commercial lime-sulphur concentrates are used, dilution may be conveniently carried down to a concentration of about 0.8 per cent. polysulphide sulphur.

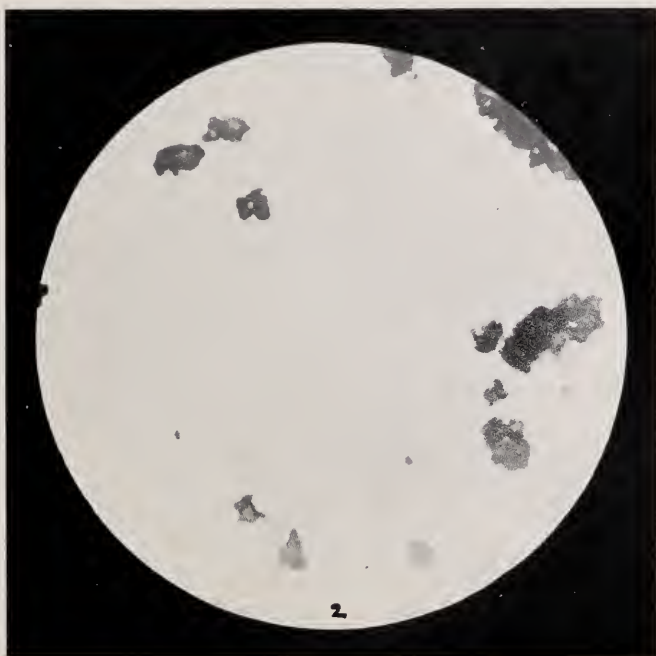
8. The sulphur dips if properly made (almost neutral to phenolphthalein) are harmless both to the sheep and to the wool. Solutions of polysulphide, at the concentration used in dipping, have no action on wool even on prolonged steeping. On the fleeces of dipped sheep polysulphide is rapidly and almost quantitatively converted into thiosulphate by atmospheric oxidation, and this occurs long before the fleeces are dry. No depilatory substances are formed in detectable amount, as intermediate products in normal decomposition. If, however, free base be present in large quantity along with polysulphide it is possible for monosulphide to be formed as intermediate product in the course of atmospheric oxidation, and the possibility of depilatory action then arises. Minor quantities of free hydroxide, up to about 10 per cent. of the total base present in the dip, appear to be of no practical consequence, since they are converted into thiosulphate during atmospheric oxidation. The depilatory action of monosulphide or hydrosulphide is much more violent than that of free hydroxide.

Under ordinary circumstances a dip would have to be very badly made indeed before the amount of residual base present could become a source of danger in practical dipping, and if reasonable care is taken in preparation the possibility of injurious action either upon the sheep themselves, or upon their wool, is altogether ruled out.

Although dyeing or spinning tests could not be carried out at this laboratory, it appears highly improbable that the expert dealer in woollen fabrics could tell the difference between a washed fleece previously dipped in the sulphur dips and a washed fleece previously dipped in any of the ordinary proprietary dips.

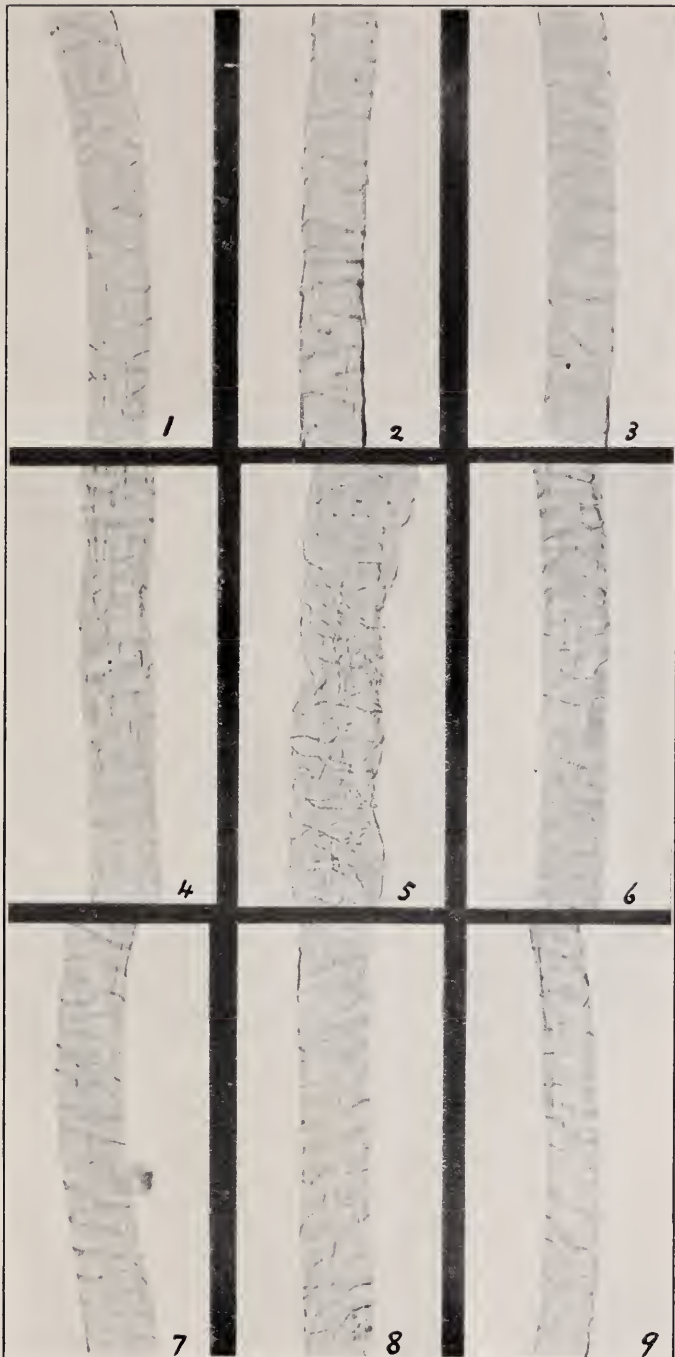


Flowers of Sulphur.



Ground Rock Sulphur, or Sulphur Flour.





**Report upon the Dipping Trials carried out
with the different Proprietary and
Home-made Sheep Dips in South Africa.**

BY

G. A. H. BEDFORD,

Entomologist, Division of Veterinary Research.



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IN view of the importance of effective dipping against scab in South Africa a series of trials upon the dips in common use was carried out at the request of the Sheep Division of the Union.

In conducting the tests the primary object was naturally that of determining the efficacy of the various preparations for the cure of scab, the subsidiary purpose being that of noting the general effect upon the sheep themselves and upon their wool.

MODE OF CARRYING OUT THE TRIALS.

(1) *Number of Sheep used for Test.*—Six sheep were taken for each trial. This number was regarded as providing a fair test, while at the same time keeping the total number of animals used in all the tests within the reasonable limit suitable for close experimental observation.

(2) *Infection of Experimental Sheep.*—Since the number of sheep in each test was limited, it naturally became of great importance to have each animal badly infected, and so to subject the dips used to a really reliable trial. Since it was found difficult to procure naturally infected sheep in a sufficiently uniform stage of disease, the process of artificial infection was adopted. For this purpose a small patch was shaved upon the back of each sheep, upon which live adult acari were then placed under protection of a glass cover in the manner described by Shilston (this Report of the Director of Veterinary Research, p. 72). After two to three days the glass cover was removed to allow the acari to spread, and after the lapse of from three to eight weeks heavy infestation was usually procured. In carrying out the tests only sheep in *very badly infected condition* were actually taken.

(3) *Type of Dipping Tank used.*—The tank used was the ordinary "Cooper's Patent Swim Bath" with a total capacity of about 225 gallons. The usual quantity of dip taken for each test was 150 gallons, this amount giving a sufficient and convenient working depth.

(4) *Policy in Testing.*—Home-made dips were prepared according to the accepted formulae. Proprietary dips were made up strictly in accordance with the vendor's instructions for use. No preliminary hand-dressing was adopted, since this practice—although admittedly a valuable adjunct to dipping—is by no means universal, and since it was desired to test the various preparations *solely as dips*. In the limited nature of the trials no attempt was made to place the various preparations in order of merit, and no attempt was made to modify

the "directions for use" supplied with proprietary preparations, except in so far as conditions of immersion and interval between successive dippings are concerned.

(5) *Immersion*.—According to the instructions issued for use with the various dips the immersion period varies from one to two minutes, although in some cases no definite immersion period is specified at all. It was, therefore, considered advisable to adopt the same immersion in all tests in order to secure comparative results. Theoretically, of course, the parasitocidal effect of a fluid is not necessarily related to the time of immersion, since the action goes on in the fleeces long after actual emergence from the tank. All that is wanted is thorough penetration of scab crusts by the fluid, and the time necessary for this varies according to the severity of infection. At the same time, exigencies of practical dipping on the large scale demand that the immersion period be as short as is *compatible with safety*. A preliminary trial with one of the proprietary dips showed that the recommended immersion interval of one minute was hardly sufficient to allow of thorough penetration in the absence of preliminary hand-dressing, whereas an immersion of two minutes appeared to be adequate. It was therefore decided to adopt two minutes as the standard time in experimental testing. In the actual process of dipping the immersion period was timed with a stop-watch, the head of each sheep being immersed twice with an interval of one minute between. Care was of course taken to handle the sheep as gently as possible so as to avoid accidental swallowing or inspiration of fluid. In spite of all ordinary precautions, however, unavoidable inspiration of small quantities into the trachea occasionally occurred with sheep so weakened by the ravages of scab that they were unable to look after themselves, and, with the weakest of the animals, death from traumatic pneumonia sometimes ensued as direct consequence. These few cases, not related to the composition of the dip as such, since they might have occurred with almost any fluid at all, are ignored in considering the action of the preparations used.

(6) *Interval between Successive Dippings*.—The theory of dipping demands two immersions within the life-cycle of the scab parasite, the first to dispose of live acari as such and the second to kill off acari subsequently hatching out from uninjured eggs before sufficient time has elapsed to allow of new eggs being laid. This is universally recognized, and all proprietary dips are sold with directions for use at two dippings. With different dips, however, different intervals between the two immersions are advised, and the time specified usually varies from two to four weeks. According to the life-history of *Psoroptes communis*, var. *ovis*, as prevailing under South African conditions, and as worked out by Shilston, and subsequently by myself (this report, p. 72 and p. 103), the interval between successive dippings should be nine days. Theoretical considerations indicate intervals of more than nine days as possibly unsafe, since opportunity is then afforded for acari—hatching out after the first dipping—to leave new eggs to survive the second dipping.

For South African conditions, therefore, a test carried out with any proprietary dip, at an interval longer than nine or ten days, would be invalid in the event of failure, and in fairness to the nature of the preparation all tests were accordingly carried out on the basis of second dipping *on the ninth day*.

In a few cases additional tests were also carried out at single immersion, dual dipping at longer interval, and at immersion at one minute instead of two.

(7) *Examination for the Cure of Scab.*—The experimental sheep were examined before, and again a few days after, each dipping in order to determine the relative abundance of live acari. After the second immersion the animals were kept under close observation in isolation stalls for six months to make sure that all acari had really been destroyed and had not merely escaped the systematic search to which each animal was subjected within a few days of dipping. In this way cure, or absence of cure, could be definitely established, since in six months redevelopment of original infection would have occurred if this had been possible.

(8) *Examination of the Skin and Wool of Dipped Sheep.*—The sheep were examined within a day or two of immersion in order to ascertain whether any damage to the skin or general health was apparent, and at the same time any obvious effect upon the wool was looked for.

Beyond general observations on the appearance of the fleeces and upon the microscopical structure of the wool-fibre no detailed tests were made. Scouring tests would doubtless have been of interest, but since any report in this direction would be of little value unless made by an authorized wool expert no attempt was made to carry them out. In any case the fleeces of the half-denuded sheep were valueless owing to the ravages of scab, and, as already mentioned, the trials were conducted with the primary object of determining the efficacy of the dips for the cure of the disease.

To avoid repetition in the text to follow it may be stated at this point that no damage either to the wool or to the sheep themselves could be definitely detected with any of the dips, except where specifically mentioned. The weakest sheep, of course, suffered a temporary setback as the result of dipping, but this could not be stated to be more definitely marked with any one preparation than with any other.

Wherever cure of scab was effected the sheep naturally improved very much in general health and condition in the ensuing weeks.

In regard to the microscopical structure of the wool-fibre, Mr. Green (of this division), on the basis of comparative examination of wool from the same sheep before and after dipping, reported that no distinct damage to the fibre could be made out in the case of any of the dips used.

RECORDS OF TRIALS.

(1) *Home-made Lime-sulphur Dip.*—In preparing this dip the formula given in Bulletin No. 3, Department of Agriculture, Union of South Africa, was adopted (*vide* also Green's report, this volume, p. 129).

Two tests were carried out, one in which a single immersion was given and the other in which a second immersion was given nine days later:—

14th March, 1914.—Twelve badly infected sheep dipped.

23rd March, 1914.—Six of these dipped again.

Result.—Single dipping proved ineffective in two cases out of the six. A few acari were found on one sheep a few days after dipping, and upon another six weeks later—probably as the result of uninjured eggs hatching out. The sheep dipped twice were found to be completely cured, and no acari could be detected within six months of the second immersion.

(2) *Capex Lime-sulphur Dip.*—This is a high-grade commercial concentrate recently placed upon the market by the Cape Explosives Company. The preparation was tested at two dilutions—one of Capex to eighteen of water, and one of Capex made up to forty with water. In neither case could live acari be found either after the first or second dipping, and observation of the sheep over six months following the second dippings showed cure to be complete in both cases. It would therefore appear that Capex is effective at a dilution so high as 1:40, i.e. $2\frac{1}{2}$ gallons of concentrate per 100 gallons of water.

(3) *Modderfontein Explosives Company's Lime-sulphur Concentrate.*—This preparation has not yet been placed upon the market, but the makers forwarded a sample for trial. It is, however, proposed to secure higher concentration before actually marketing as a dip.

The dilution tested was one of concentrate to twelve of water. Cure of scab was effected with the usual two dippings.

(4) *Home-made Caustic Soda and Sulphur Dip.*—This dip was also made up according to the directions given in Union Bulletin No. 3 already referred to (*vide* also this Report of the Director of Veterinary Research, p. 116). Twelve badly infected sheep were dipped on 16th March, and six of these again on 25th March. Five out of the six sheep, receiving only one dipping, were cured of scab, the remaining animal showing live acari three weeks later. The sheep dipped twice were all cured. The necessity for the second dipping is here illustrated, but it is interesting to note that the first dipping effected the major destruction, although a second dipping was required to catch the few acari which in one case hatched out from undamaged eggs.

(5) *O'Gorman's Sulphur Dip.*—This is a "caustic soda and sulphur" concentrate, although, as will be seen from Green's report, it is not of particularly high grade. The dilution recommended was one gallon of drum fluid to thirty gallons of water. Six badly infected sheep were dipped on 7th July, and again on 16th July. Within a few days of the second dipping one sheep still showed acari, so that the dip cannot be regarded as completely effective. Green explains this upon the relatively low content in "polysulphide sulphur" at the dilution recommended, and it is therefore probable that if a tank strength of 1 gallon of drum fluid per 15 to 20 gallons of water were used the preparation would be as effective as the home-made caustic soda and sulphur dip.

(6) *Home-made Loogas and Sulphur Dip.*—This dip was also made according to the formula of Bulletin No. 3. For information as to composition of this dip reference may be made to p. 139 of this report.

Six infected sheep were dipped on 23rd September, and again on 2nd October. A few acari were found on two out of the six sheep within a week of the first dipping, but at no time after the second dipping could acari be found. The sheep were therefore cured of scab.

(7) *Little's Fluid Dip*.—Dipping was carried out according to vendor's concentration on 1st July, and again on 10th July. No living acari could be found either after the first or the second immersion, and, as subsequent examination showed, cure of scab was complete.

(8) *Haywood's Paste Dip*.—In accordance with directions for use, one tin of paste was stirred up with 2 gallons of water and diluted to 125 gallons in the dipping tank. For the second dipping a lower concentration was used, 200 gallons of water being used instead of 125 gallons. No live parasites could be found after either dipping, and cure of scab was duly established. It may be mentioned that this dip was one recommended for use at immersion of a "full minute"; but for reasons given earlier the usual two minutes period was adopted.

(9) *McDougall's Powder Dip*.—With this preparation also, in the recommended proportions of one packet of dip per 25 gallons of water, effective cure of scab was established. Although two out of the six sheep were still fairly badly infected after the first dipping no acari could be found at any time after the second.

(10) *Cooper's Dipping Powder*.—Owing to the very wide use of this preparation three tests were carried out:—

- (a) Under conditions parallel to the other tests. Powder at the rate of one packet per 25 gallons of tank fluid required was used in the usual way, i.e. made into a paste a few hours before use and then stirred into the bulk of water in the swim bath. Six sheep were dipped for two minutes on 11th August, and again on 20th August. No live acari could be found after either immersion, and observation of the animals for six months after the second dipping showed cure to have been effected.
- (b) In strict accordance with packet directions. At the request of the makers, who claimed that their "directions for use" were based upon a world-wide experience, Cooper's Dip was also tested at fourteen-day interval, one minute immersion, and half strength at the second dipping. Of the six sheep dipped, cure was effected in five cases, but one animal escaped cleansing and showed live acari about a month after the second dipping.
- (c) At two minutes' immersion, but at fourteen days' interval. Six sheep were dipped in full strength at the first dipping, and, since they carried a few months' wool, at half strength the second dipping. No live acari could be detected after either dipping, and cure was duly established.

These tests, which do not claim to be exhaustive, nevertheless bring out one point, that dipping for one minute at fourteen-day interval is not absolutely effective. The failure in test (b) may be due either to infection remaining under a crust not penetrated by immersion for one minute, or it may be due to the dipping interval being beyond the ten day life-cycle of the parasite. The fourteen-day interval is theoretically long enough to allow hatching out of uninjured eggs after the first dipping and relaying of new eggs before the second. Considering that acari only reappeared on one sheep a month after the second dipping we are inclined to attribute redevelopment of infection to this latter cause. In test (c) all sheep were cured,

although the only difference was the time of immersion and although the interval was still beyond the life-cycle of Psoroptes. This may possibly be due to more complete destruction of acari at the first dipping or to chance (fickle when small numbers are concerned), ordaining that no fresh eggs should be laid within fourteen days of the first dipping. The determination of this point would have involved more experimental time and animals than we could afford to give, but the fact remains that two dippings at fourteen-day intervals and one minute immersion cannot be relied upon, and theoretically any interval longer than nine days must be regarded as unsafe, more particularly when the number of infected sheep becomes large and the chance of redevelopment of original infection correspondingly greater. Opportunity for any acari protected by hard crusts to escape destruction at the first dipping is of course much diminished by the auxiliary hand-dressing recommended in the directions for use supplied with this dip.

(11) *Home-made Arsenite of Soda and Sulphur Dip*.—This dip was made up at the rate of $2\frac{1}{4}$ lb. of arsenite of soda and 5 lb. of sulphur per 100 gallons of tank fluid. The arsenite of soda was dissolved in a few gallons of water, the sulphur separately mixed into a paste with water, and both then stirred into the requisite amount of water in the dipping tank. Two tests were carried out at one and two minutes' immersion respectively. Four days after the first dipping acari were found on four out of the six sheep in the former case, and on only one in the latter. After the second dipping, however, complete cure was effected in both cases.

(12) *Jeyes' Fluid*.—This was used at tank strength of 1 gallon of Jeyes to 100 gallons of water. No live acari could be found after either first or second dipping, and final cure was established by subsequent inspection. In connection with the use of this fluid the observation of Shilston is of interest. He found that solutions of 1 in 100 to 200 were fatal to the acari, but had little effect upon the eggs. At the same time he found that a few sheep dipped in Jeyes' Fluid were completely cured of scab after a single immersion. It would therefore appear that sufficient disinfectant remained in the fleeces to dispose of fresh acari hatching out from uninjured eggs. In spite of this, however, a single immersion should not be relied upon, and a dip found efficient under such conditions on the small scale would have to be tested upon a very large scale before the single dipping could be regarded as safe.

(13) *Kerol*.—One gallon per 100 gallons of water. A week after the first dipping two out of the six sheep still showed a few acari, but after the second dipping definite cure was established.

(14) *Leach's Sheep Dip*.—According to the directions for use, 4 gallons of fluid per 40 gallons of tepid water, finally diluted to 160 gallons with cold water in the tank, was permitted. Of six infected sheep dipped on 23rd March at this concentration, one died on the day of dipping, three the day following, and one more on the third day. The one remaining sheep, together with six clean sheep, were then dipped on 1st April.

With two of the clean sheep special care was taken to prevent immersion of the head, and so to eliminate the possibility of accidental

swallowing of the dip. Of the seven sheep five died within three days, including the two animals whose heads were not immersed. The remaining two survived. One of these was the originally infected sheep which had resisted both dippings, and in this case cure of scab was recorded.

Post-mortem examination and analysis of residual urine in the bladder pointed strongly to cresolic and phenolic intoxication. The two healthy sheep succumbing in spite of non-immersion of the head suggests absorption through the skin. We regret having to record this case, since theoretical considerations of the nature of the dip render it highly probable that at lower concentrations the preparation might be both effective and harmless. At the same time it is our definite policy to test proprietary preparations only in accordance with vendor's instructions, unless specially requested to try alternative recommendations. It may also be added that no definite immersion period was specified, although we do not think that a shorter immersion at the same high concentration would have saved our animals, since absorption from the soaking fleeces may easily go on for some time after emergence from the tank.

Incidentally, Leach's dip was recommended for use at a dipping interval of three to four weeks.

(15) *McDougall's Tobacco Dip*.—One tin of extract was used per 150 gallons of water. No live acari could be found either after the first or the second dipping, and effective cure was finally established in the usual way.

(16) *Dreadnought Tobacco Dip*.—One tin of extract was used per 175 gallons of water. The results were the same as with McDougall's Tobacco Dip.

(17) *Magic Sheep Dip*.—This is a tobacco dip in powder form, made by Hunter & Gow, of Liverpool, and obtainable through Mayhew & Noton, their Durban agents. The mode of preparation is cumbersome, and this perhaps militates against its popularity, since customers expect convenience in use as the main feature of a proprietary article.

In making up the tank fluid 100 lb. of the powder was steeped in 16 gallons of warm water and left over-night. In the morning the extract was made up to 150 gallons in the tank. In making up the fluid for the second dipping the decoction was stewed for a few hours before using.

The dip was found to be effective in the cure of scab. A few acari were found on one sheep after the first dipping, but none could be detected at any time after the second.

(18) *Home-made Tobacco Dip*.—30 lb. of Transvaal tobacco leaf was steeped over-night in a large pot containing 30 gallons of water. In the morning it was brought to boiling point, allowed to simmer for a few minutes, cooled somewhat, strained through a sack, and the extract made up to 150 gallons in the dipping tank.

No acari could be detected on any of the sheep either after the first or the second dipping, and complete cure was duly established by subsequent observation.

All the tobacco dips were observed to stain the wool slightly, but it seems not improbable that the discoloration would be removed in the scouring process.

SUMMARY AND CONCLUSIONS.

Practical trials on a small scale were carried out with the following sheep dips available in South Africa:—

(1) Home-made Lime Sulphur Dip, (2) Capex Lime-sulphur Concentrate, (3) Modderfontein Lime-sulphur Concentrate, (4) Home-made Caustic Soda and Sulphur Dip, (5) O'Gorman's Liquid Sulphur Dip, (6) Home-made Loogas-Sulphur Dip, (7) Little's Fluid Dip, (8) Hayward's Paste Dip, (9) McDougall's Powder Dip, (10) Cooper's Sheep Dipping Powder, (11) Arsenite of Soda and Sulphur Dip, (12) Jeyes' Fluid, (13) Kerol, (14) Leach's Sheep Dip, (15) McDougall's Tobacco Dip, (16) Dreadnought Tobacco Dip, (17) Magic Sheep Dip, (18) Home-made Tobacco Dip.

From six to twelve sheep, very badly infected with scab, were used in each test. In the main series of trials two dippings at an immersion period of two minutes, and a dipping interval of nine days, were carried out—the nine-day interval having been previously established as just within the life-cycle of *Psoroptes communis*, var. *ovis*, in South Africa.

The different batches of dipped sheep were tested for eradication of scab by being kept under observation in isolation stalls for six months after dipping.

The general results showed successful cure of scab in all trials except one. The exception is represented by O'Gorman's dip, but the failure in the case of one sheep is doubtless due to too high a dilution of the drum fluid as recommended in the directions for use. At higher tank strength this dip would also be effective.

No attempt was made to place the various preparations in order of merit. This would have involved more extensive trials on a larger scale, and in any case it was not desired to draw invidious distinction between different proprietary articles.

A few tests carried out at shorter immersion period and longer dipping interval indicated that, although such conditions might be effective, they could not be regarded as safe—especially in the absence of auxiliary hand-dressing, where time must be allowed for penetration of scab crust and where the danger of development of original infection is greater.

The effect of the various dips upon the general health of the sheep, and upon the skin and wool, was not observed to be unfavourable, except in the case of Leach's dip, where obvious intoxication occurred in ten out of the twelve sheep dipped. The deaths occasioned in this case are attributed to the high concentration recommended for use. At lower concentration of tank fluid and with revised instructions for use this dip would probably be efficacious and innocuous. In regard to the recent controversy concerning the home-made sulphur dips there is every indication that these, if properly used, are both effective and harmless.

As final conclusion it may be safely asserted that, with the exceptions indicated, *all the dips tested are permissible and effective in the cure of scab if properly used at an immersion period of two minutes and a dipping interval of nine days. Single dipping or dual dipping at a longer interval than nine days, or immersion of less than two minutes, may be effective in some cases, but cannot be relied upon.*

The tests recorded are to be regarded as experimental in nature, and as serving as basis for more extensive trial in the field. The most interesting point in the laboratory trials is the apparent ease with which scab can be cured with almost any accredited dip.

Upon the Composition and Analysis of Polysulphide Solutions.

BY

HENRY H. GREEN,

Biochemist to the Division of Veterinary Research.

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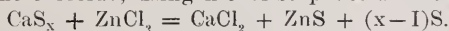
THE work involved in the preceding paper upon the sulphur sheep dips was taken up early in 1914 and, in substance, read at the meeting of the Transvaal Veterinary Association in August of that year. While the work was in progress, and in the interval between the date of its communication and the date of writing up for publication in the present report of the Director of Veterinary Research, several papers have appeared dealing with the composition of lime-sulphur washes. Those to which it is proposed to refer are the two papers by Ramsay in the *Journal of Agricultural Science* for May, 1914, and December, 1914, and the paper by Auld in the *Journal of the Chemical Society* for April of this year (1915).

Certain references to the literature of the subject are made in these papers, but unfortunately we have been unable to procure any but a few of the relevant originals in any of the available South African libraries. The discussion must therefore be confined to those which have been obtained, and proceed upon the assumption that the more recent authors have adequately summarized the earlier work which they quote.

The attempt to determine the nature of the polysulphides actually present in a solution prepared by boiling lime and sulphur, or caustic soda and sulphur, with water, is of course dependent upon the methods of analysis adopted, and it is therefore proposed to first devote brief consideration to these. Exigencies of other work have prevented an elaborate trial of all the theoretically possible analytical methods, but a number of general observations may still be of interest.

The old methods for the analysis of lime-sulphur solutions adopted by the *U.S. Association of Official Agricultural Chemists* previous to 1911 are given in the last edition of Wiley's *Agricultural Analysis*, and need not, therefore, be detailed in full. In the proceedings of the Twenty-ninth Annual Convention of the Association (Washington, 1913), which publication represents the last report available at the moment of writing, S. D. Averitt, referee on insecticides for the year, discusses the old methods and compares them with those tried in 1912 based upon the paper by J. E. Harris.¹

In the old methods the "monosulphur equivalent," or base present in combination in sulphide and polysulphide form, irrespective of the nature of the sulphide, is determined by titration with N_{10} ammoniacal zinc chloride, using nickel sulphate as external indicator.

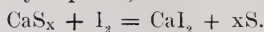


Thiosulphate is then determined in the filtrate (or its equivalent) from the zinc precipitate, by titration with N_{10} iodine after neutralization of ammonia. After iodine titration, i.e. conversion of thiosulphate to tetrathionate and any trace of sulphite to sulphate, the sulphur

¹ Michigan Agricultural College, Technical Bulletin No. 6, 1911.

originally present as "sulphate and sulphite" is determined by precipitation with barium chloride in the cold. Total sulphide sulphur is then determined directly on the ammoniacal zinc precipitate or, more usually, indirectly as the difference between total sulphur and sulphur present as thiosulphate and sulphate.

In the new methods the monosulphur equivalent is determined by direct titration with $N/10$ iodine to the point of disappearance of the yellow colour due to polysulphide,



Thiosulphate is then determined by continuing the titration to re-appearance of yellow colour due to free iodine, or to iodine end-point to starch. The sulphur is then filtered off and "sulphate and sulphite" determined in the filtrate, while the total sulphide sulphur may either be found by difference or by weighing the sulphur filtered off—direct or after conversion into barium sulphate.

In general analytical practice the main difference, according to Averitt, is that the 1911 methods show a lower proportion of thiosulphate sulphur and a correspondingly higher proportion of sulphide sulphur. Acting upon the assumption that no derivative higher than the pentasulphide is capable of existing in solution, and that all the calcium is present as polysulphide, thiosulphate, and sulphate, Averitt comes to the conclusion that the 1911 methods are inaccurate and the 1912 methods reliable. He determines the total calcium present by the usual oxalate method, and finds calcium determined by the 1912 methods as the sum of the fractions present as sulphide (monosulphur equivalent), thiosulphate, and sulphate, to be nearer the true total calcium than if determined by the 1911 methods. He further finds that the ratio of "monosulphur equivalent" to "total sulphide sulphur" is frequently higher than 1:5 when determined by the 1911 methods, and for this reason also pronounces the original methods less accurate than those of 1912.

McDonnell, in a paper upon the "Composition and Methods of Analysis of Lime-Sulphur Solution," printed in the same report in the pages immediately following Averitt's discussion, criticizes Averitt's findings, and maintains the correctness of the 1911 methods.

From the limited experience we have had of the two methods we are in agreement with McDonnell, but since our reasons for regarding the old methods as the more accurate are not quite the same as his, a further consideration of the question is perhaps of interest.

The determination of total sulphur need not be considered, since this estimation is simple and liable to very little error. Various analysts have considered the advisability of using sodium peroxide in preference to caustic soda and hydrogen peroxide as oxidizing agent, but since we have never detected any real difference in the results obtained by minor variations in the mode of oxidation it is unnecessary to discuss the matter.

The real difference between the 1911 and the 1912 methods concerns the monosulphur equivalent, the thiosulphate and the sulphate. We have compared both methods and, although agreeing that the thiosulphate determination by the new method is considerably higher than by the old, we have never detected the large differences which Averitt records, nor have we found so great a discrepancy between the total calcium oxide as actually determined by the oxalate

method and the total calcium oxide determined by summation of constituents estimated by the 1911 methods.

The following three analyses—one of a diluted commercial concentrate, one of a laboratory prepared sample a week old, and one of a laboratory prepared sample after storage in a stoppered bottle for over a year—are typical of our own results. In the estimation of monosulphur equivalent by the 1911 methods a few c.c. of 10 per cent. ammonium chloride were first added to 20 c.c. of solution to be titrated (plus 50 c.c. water) in order to facilitate flocculation of the ammoniacal zinc precipitate, and the thiosulphate was then determined in the filtrate after rapid washing with considerable quantities of water. The total calcium oxide was found by titration of precipitated oxalate with $N/_{10}$ permanganate. The results are stated directly per 100 c.c. of the diluted solutions.

Sample.	Methods.	Total sulphur.	Sulphide-S.S. (difference.)	Thiosulphate-Sulphur.	Mono-sulphur equivalent M.S.	sulphur as sulphate and sulphite.	Total CaO.		Ratio M.S.:S.S.
							Determined.	Computed.	
1	1911	1.001	0.965	0.035	0.191	0.001	0.369	0.367	1:5.05
	1912	"	0.948	0.045	0.189	0.008	"	0.384	1:5.02
2	1911	1.235	0.994	0.239	0.195	0.002	0.557	0.554	1:5.10
	1912	"	0.968	0.259	0.191	0.008	"	0.575	1:5.07
3	1911	1.380	1.048	0.330	0.220	0.002	0.680	0.677	1:4.72
	1912	"	1.021	0.350	0.217	0.009	"	0.702	1:4.70

From these results it would appear that the two methods give substantially the same results, the main difference being an appreciably higher value for thiosulphate and sulphate by the 1912 methods. The data for calcium oxide, however, give better agreement by the 1911 methods than by those of 1912.

Six further samples of lime-sulphur solution, tested by both methods, gave fairly concordant results, but in all cases, except one, a slightly lower monosulphur equivalent was registered by the iodine titration than by titration with ammoniacal zinc. This difference was about 0.3 c.c. in a burette reading of 25 c.c. In one case the iodine titration was rather higher, but the sample was open to suspicion of containing free lime, since it gave a titration figure to $N/_{10}$ acid (methyl orange) well above the $N/_{10}$ ammoniacal zinc reading. In this case also the computed calcium oxide checked better with the estimated total calcium oxide when the 1912 methods were used, whereas in the other five cases computation by the 1911 methods gave the closer agreement. On the whole it appeared that the 1911 methods gave a computed calcium oxide value about 1 per cent. too low and the 1912 methods about 3 per cent. too high.

The thiosulphate by the 1912 methods was always rather higher, but the difference between the two methods never exceeded about 1 c.c. in a titration of 10 c.c.

The sulphate (and sulphite) by the 1911 methods never amounted to more than a trace, whereas by the 1912 methods it was always appreciable. This difference in the sulphate estimation, although very small when compared with the total sulphur present, appears important to us, since it suggests an oxidation by iodine as a minor

as thiosulphate by the 1912 methods. Stating the results in the form of sulphur we have approximately:—

- | | |
|----------------------------|-------------------------------------|
| (1) Monosulphur equivalent | $23.1 \times 0.0016 = 0.037$ gm. S. |
| Thiosulphate | $1.4 \times 0.0064 = 0.009$ gm. S. |
| (2) Monosulphur equivalent | $21.7 \times 0.0016 = 0.035$ gm. S. |
| Thiosulphate | $5.8 \times 0.0064 = 0.037$ gm. S. |
| (3) Added to (2) as H_2S | $3.1 \times 0.0016 = 0.005$ gm. S. |

The relatively small difference in the monosulphur equivalent and the large difference in the "thiosulphate sulphur" effected by the presence of relatively small amounts of sulphuretted hydrogen is clearly brought out. It may be added that not only is the decolorization end-point lowered in presence of free sulphuretted hydrogen, but that it is also more difficult to detect. In presence of larger quantities of hydrogen sulphide the end-point is exceedingly difficult to determine, while the final end-point to excess iodine (starch) is too low, probably as a result of escape of gas during titration.

The "monosulphur equivalent" as found by titration with ammoniacal zinc is, of course, *raised* in presence of free sulphuretted hydrogen in precise proportion to the amount added. The free ammonia in the reagent converts the free hydrogen sulphide into ammonium sulphide which then precipitates as zinc sulphide in the usual way. 20 c.c. of solution A + 50 c.c. of water, titrated to $N/_{10}$ am. $ZnCl_2$, gave a reading of 23.3 c.c. After adding 5 c.c. H_2S solution (= 3.1 c.c. $N/_{10}$ H_2S) gave 26.5 c.c., thus showing a quantitative registration of added sulphide. The thiosulphate determination in the filtrate remained unaffected.

A few tests carried out with addition of small quantities of calcium monosulphide and calcium hydrosulphide, prepared by mixing lime-water and sulphuretted hydrogen in calculated proportions, showed analogous results. Added monosulphide titrated practically quantitatively both to am. zinc and to iodine. Added hydrosulphide behaved just as a mixture of monosulphide and free hydrogen sulphide, raising the zinc titration without affecting the thiosulphate determination and lowering the iodine end-point with consequent raising of the apparent thiosulphate value.

In passing, it may be mentioned that calcium monosulphide can be titrated almost quantitatively with iodine by making use of the yellow polysulphide end-point. The first few drops of iodine result in the formation of yellow polysulphide owing to re-solution of precipitated sulphur by excess of monosulphide. This polysulphide colour persists and finally disappears just before the end-point to starch. At the same time traces of sulphate appear to be formed by subsidiary oxidation. The presence of calcium monosulphide or "hydroxy hydrosulphide" in a lime-sulphur solution would therefore appear to make little difference to the iodine titration, although the presence of sulphuretted hydrogen, or its equivalent hydrosulphide, does seriously interfere.

In presence, then, of free hydrogen sulphide, erroneous results are obtained by *both* 1911 and 1912 methods. The error by the latter method is much more serious, since it affects the "thiosulphate" and is therefore quadrupled by calculation. We have, however, never been able to definitely detect the presence of hydrosulphide or free sulphuretted hydrogen in lime-sulphur solutions. According to theoretical expectation, simple titration to $N/_{10}$ acid, with methyl

orange as indicator, should give the total base present in all forms other than thiosulphate, sulphate, and sulphite. Comparison of an acid titration with an am. zinc titration should therefore reveal the presence of free hydrogen sulphide on the one hand or free calcium hydroxide on the other if either of these is present in a polysulphide solution. If the two titrations are the same the solution theoretically only contains sulphide (together with thiosulphate of course). If the acid titration is higher free lime is suggested, whereas if the zinc titration is higher free sulphuretted hydrogen or calcium hydrosulphide is indicated. We have, however, carried out about a dozen comparative titrations with different samples of lime-sulphur solutions, but have invariably found the acid titration to give a slightly higher reading, and we therefore consider that traces of free lime are usually present. The differences appear to be very small—in solution A above, the acid titration gave 23.5 c.c. against a zinc titration of 23.3 c.c.

If *free lime* is present the difference between the 1911 and 1912 methods is very much more marked, but takes a different form. In the zinc titration a slightly higher monosulphur equivalent is registered, but this may be largely counteracted by the preliminary addition of excess of ammonium chloride to replace lime by ammonia. The bulk of the free hydroxide passes into the filtrate, and can be detected there by a higher alkalinity than that derivable from the free ammonia in the zinc reagent. In the iodine titration by the 1912 method part of the lime registers as monosulphur equivalent, part possibly as thiosulphate, part appears as sulphate in combination with oxidized sulphur, and part may remain unchanged. The following titrations on solution A (above) indicate the behaviour:—

$$(1) \text{ 20 c.c. A + 50 c.c. water to } N_{10} \text{ am. zinc} = 23.3 \text{ c.c.}$$

$$(2) \text{ 25 c.c. lime water alone + 50 c.c. water to } N_{10} \text{ acid} = 11.0 \text{ c.c.}$$

$$(3) \quad \text{,} \quad \text{,} \quad \text{,} \quad \text{,} \quad N_{10} \text{ I}_2 = 1 \text{ c.c. (approx.)}$$

$$(4) \text{ 20 c.c. A + 25 c.c. lime water + 50 c.c. water to } N_{10} \text{ zinc} = 24.7 \text{ c.c.}$$

$$\text{thiosulphate in filtrate to } N_{10} \text{ I}_2 = 1.3 \text{ c.c.}$$

$$(5) \text{ 20 c.c. A + 25 c.c. lime water + 50 c.c. water + 10 c.c. 10 \% NH}_4\text{Cl titrated to } N_{10} \text{ zinc} = 23.6 \text{ c.c.}$$

$$\text{thiosulphate in filtrate to } N_{10} \text{ I}_2 = 1.3 \text{ c.c.}$$

$$(6) \text{ 20 c.c. A + 50 c.c. water to } N_{10} \text{ I}_2 \text{ to decolorization} = 23.0 \text{ c.c.}$$

$$\text{to excess iodine} = 24.5 \text{ c.c.}$$

$$\text{hence "thiosulphate"} = 1.5 \text{ c.c.}$$

Now tested and found slightly acid = 0.2 c.c. N_{10} NaOH.

Now filtered and precipitated with BaCl_2 gave 0.0005 gm. S.

$$(7) \text{ 20 c.c. A + 50 c.c. water + 25 c.c. lime water to } N_{10} \text{ I}_2 \text{ to decolorization} = 30.3 \text{ c.c.}$$

$$\text{to excess iodine} = 32.2 \text{ c.c.}$$

$$\text{hence "thiosulphate"} = 1.9 \text{ c.c.}$$

Now still alkaline and to N_{10} HCl = 1.1 c.c.

Now filtered, precipitated with BaCl_2 gave 0.04 gm. BaSO_4 = 0.0055 gm. S.

$$(8) \text{ 20 c.c. A + 50 c.c. water + 25 c.c. lime water titrated to decolorization, then tested and found alkaline, titrated to } N_{10} \text{ HCl} = 3.0 \text{ c.c.}$$

The following data indicate the *formation of acid* during iodine titration. The solution used was the diluted concentrate A, mentioned earlier. After final thiosulphate end-point to starch one drop of $N/_{10}$ thiosulphate was added to discharge the blue colour and the solution then titrated to $N/_{10}$ NaOH.

Aliquot.	c.c. Water added.	c.c. $N/_{10}$ I_2 to colourless.	c.c. $N/_{10}$ I_2 to starch.	Difference = thiosulphate in c.c. $N/_{10}$ I_2 .	Acidity after final end-point in c.c. $N/_{10}$ NaOH.	Sulphate.
20 c.c.	10	23.2	24.4	1.2	Neutral	—
"	50	23.0	24.6	1.6	0.2	0.0005 gm. S.
"	100	22.7	24.5	1.8	0.5	—
"	150	22.4	24.6	2.2	0.7	—
"	300	21.3	24.6	3.3	1.4	0.0011 gm. S.

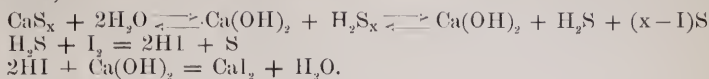
It is seen that as the dilution rises the monosulphur equivalent decreases, with a proportionate rise in the so-called thiosulphate—behaviour not shown when titration is carried out with ammoniacal zinc chloride. At the same time an increasing acidity is developed and a slight increase in the amount of sulphate can be detected. The lowered monosulphur equivalent could be wholly attributed to error in detecting the true decolorization end-point, if it were not for the fact that at low dilutions a practically neutral final reaction is shown, while at high dilutions a marked acidity is evident. This final acidity is presumably due to hydriodic acid, corresponding to free hydrogen sulphide at the colourless end-point. That free sulphuretted hydrogen is present at the first end-point, can also be shown by testing a filtered portion of the liquid. Towards neutral nickel sulphate no sulphide reaction is shown, but towards alkaline nickel sulphate or lead acetate a sulphide reaction is distinct. It may also be shown by adding phenol phthalein to the solution just before the first end-point is reached. The pink colour due to hydrolysis of residual polysulphide goes just before the colourless end-point is reached, thus suggesting the presence of hydroxysulphide or free sulphuretted hydrogen. If now a drop of methyl orange be added the reaction is observed to be alkaline (hydroxysulphide registering its base towards this indicator), but to become acid during the subsequent titration for thiosulphate, thus indicating the conversion of sulphuretted hydrogen into hydriodic acid.

Since the final acidity is more marked at high dilutions than at low it must arise as a bye-product of the iodine titration itself. If due to initially present sulphuretted hydrogen, calcium hydro-sulphide, or hydropolysulphide, it should be independent of the dilution and should be registered to ammoniacal zinc as well as to iodine. No simple process of hydrolysis on dilution is alone sufficient to account for the formation of sulphuretted hydrogen unbalanced by its equivalent of calcium hydroxide. The suggestion therefore is that it arises by oxidation, and this idea is borne out by the slight increase in sulphate noted at high dilution.

The major reaction with iodine,

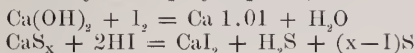


presumably occurs not directly, but indirectly with hydrolytic products,

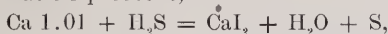


As fast as hydrolytic sulphuretted hydrogen is formed it is removed by iodine and sulphur is precipitated. The hydriodic acid combines with hydrolytic hydroxide, and so the main reaction goes on.

At the same time it is possible that at high dilution part of the hydrolytic hydroxide reacts directly with iodine and part of the hydriodic acid directly with polysulphide,



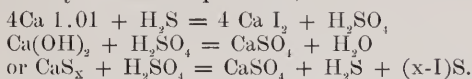
If now oxidation proceeds,



the main result is unaffected and still remains, in essence



But if secondary oxidation proceeds,



the balance is disturbed and free sulphuretted hydrogen may accumulate at the first end-point or free hydriodic acid at the second. Uncombined sulphur at the instant of separation might also suffer oxidation by iodine in alkaline medium, just as free sulphur is oxidized by bromine. The net result would be much the same. The "monosulphur equivalent" would be lowered, firstly, as the result of removal of polysulphide calcium in the form of sulphate, and, secondly, as the result of the bleaching action of the sulphuretted hydrogen liberated. The latter constituent, together with the "titration depression," would be registered as thiosulphate, and in calculation the error would be multiplied by four. With all the samples we have examined the differences in the results obtained by the 1911 and 1912 methods can be explained upon this supposition of subsidiary oxidation.

To the error introduced in this way must be added the simple titration error involved in the difficulty of detecting the decolorization end-point itself. The tendency is usually to accept decolorization at about 0.2 c.c. before it actually occurs. This error also tends to lower the monosulphur equivalent and to raise the thiosulphate. Indeed, McDonnell (*l.c.*) maintains that half the discrepancy between the two methods can be explained upon the titration error alone.

We have also repeated McDonnell's observation that added thiosulphate can be quantitatively recovered by the 1911 methods, and therefore agree that there is no reason to suppose that thiosulphate originally present is lost in the process of determination.

In regard to Averitt's contention that total calcium oxide as determined by calculation from the 1911 methods does not agree so well with the true total calcium oxide (estimated as oxalate) as it does when computed by the 1912 methods, we can only say that within our limited experience this has not been the case. Provided that titration to $\text{N}/_{10}$ acid (methyl orange) and titration to $\text{N}/_{10}$ ammoniacal zinc

gave substantially the same reading, thus eliminating hydroxide on the one hand and hydrosulphide on the other, we always found that the total calcium oxide computed by the 1911 methods agreed reasonably well with that determined by titration of precipitated oxalate with $N/_{10}$ permanganate. The extraordinary results given by Averitt (*l.c.* pp. 31, 32), in which the calculated calcium determined by the 1911 methods is about 16 per cent. below that actually found by the oxalate method, are difficult to understand. The difference is too great to be accounted for as free hydroxide, or even as oxypoly-sulphide. Incidentally, however, it may be remarked that the co-operative results (p. 33) show an extraordinary disagreement in the results of different analysts using the same methods (1912) on the same sample of lime-sulphur solution. The monosulphur equivalent varies from 1.44 per cent. to 1.63 per cent., representing a difference in burette reading of 2 c.c. in a total of 16 c.c., which is much greater than we have ever noted between the 1911 and the 1912 methods themselves. The thiosulphate varies from 2.19 per cent. to 2.91 per cent., which is again greater than the difference usually found between the two methods. The calcium oxide determined *directly* by oxalate varies from 4.54 per cent. to 5.26 per cent. In face of such conflicting data it is obvious that further detailed work is required before adopting the 1912 methods as official. We ourselves are satisfied that the original zinc titration method gives the more accurate results, and suggest that an acid titration to methyl orange be always carried out to control the presence of free lime or free sulphuretted hydrogen.

The argument that an analysis must be wrong simply because the ratio of "monosulphur equivalent" to "sulphide sulphur" is wider than 1:5 appears to us to be invalid, since it is based upon the assumption that the pentasulphide is the highest derivative capable of existing, and that a higher ratio can therefore not occur. But in freshly made solutions the ratio determined *either* by the 1911 or the 1912 methods is commonly well above 1:5, and is often as high as 1:5.4. Whether this is due to a polysulphide above the penta-, or to sulphur held in physical solution, or to hydropolysulphides of the type $\text{Ca}(\text{HS})_x$, it is difficult to say. It is true that on long standing in the cold the excess of sulphur above that required for the pentasulphide is generally deposited, but this need not necessarily be the case. The belief in the 1:5 ratio appears to rest upon the work of Divers in 1884, and it is therefore desirable that the point should be reinvestigated. It is, however, a noteworthy fact that titration of a polysulphide solution to ammoniacal zinc does not result in the precipitation of zinc sulphide or of free sulphur until about one-third of the total titer has been added, and also that on titration to iodine or acid a small but appreciable amount of reagent can be added before precipitation of sulphur begins, even when the ratio of monosulphur equivalent to total sulphide sulphur is already 1:5. According to the reaction,



the precipitation of sulphur should begin at once. It does not, and even in the cold a higher ratio than 1:5 may therefore exist. In this connection the experiments of Auld² are of interest, in which

² *J.C.S.*, April, 1915, p. 495.

evidence is adduced for the possible existence of a heptasulphide. This point will be referred to again.

The two papers by Ramsay may now be commented upon. In the first paper³ lime-sulphur solutions are regarded as consisting of a mixture of "calcium hydroxyhydrosulphide, calcium thiosulphate, calcium sulphate, with sulphur held in physical solution" (p. 201). This view is *prima facie* untenable and can be negatived by a simple test of immediate alkalinity towards phenol phthalein, but since it apparently arose from a misconception of the significance of the term "monosulphide sulphur," as used by Harris (*l.c.* 1), nothing further need be said. In the second paper,⁴ Ramsay considers it "unlikely that calcium pentasulphide is present" (p. 478) in a lime-sulphur solution, and regards the dominant derivative as the disulphide. Hydroxyhydrosulphide is still regarded as present in minor amount, with excess sulphur in physical solution. Ramsay notes that the "so-called polysulphidal sulphur" is loosely held and soluble in benzene and carbon disulphide. He determines the sulphur extracted by repeated shaking up with carbon disulphide, and states this as "free sulphur." He then finds that the unextracted sulphur, calculated against the calcium in combination with sulphur (monosulphur equivalent), corresponds roughly to calcium disulphide, and thence concludes that the latter compound is the main sulphide in solution. The unextracted sulphur and monosulphur equivalent are, however, not quite in disulphide ratio, and hydroxyhydrosulphide (monosulphide) is therefore assumed to be present. We fail to see any real basis for Ramsay's assumptions. The fact that so much sulphur is extractable by carbon disulphide is no evidence against the presence of higher sulphides, since decomposition is certainly involved in the process of extraction. In carrying out Ramsay's shaking-out method it is noticeable that extraction does not proceed as would be expected, but that sulphur is *precipitated* before it is dissolved by the solvent employed, and that precipitation is out of all proportion to the amount of carbon disulphide added. A single drop, or even the entrance of the vapour into the separating funnel, is sufficient to produce a comparatively copious precipitation of sulphur. We have also repeated Ramsay's method of extraction, but have failed to obtain the concordant results claimed for it. Incidentally, the repeated shaking with large volumes of air in the separating funnel results in the formation of small quantities of thiosulphate. Our own few trials were unsatisfactory, but in view of the detailed work of Auld (*l.c.* 2) they need not be quoted. In any case, even if Ramsay's contention that the residual proportion of sulphur corresponds to that required for the disulphide were correct, it would only mean that a derivative containing that proportion of sulphur was stable in presence of carbon disulphide, and *not* that the disulphide was *originally present*.

Auld's data are less open to objection than Ramsay's, since his extractions were carried out in absence of air. Auld finds that sulphur is *continuously* removed by sulphur solvents, and that after forty-seven hours' extraction with benzene, 95.7 per cent. of the polysulphide sulphur is recovered.

It may also be mentioned that Ramsay's reasons for assuming that the pentasulphide is absent are invalid. The figurative statement

³ *J. Agric. Sci.*, vi, 2, May, 1914.

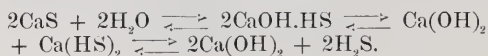
⁴ *J. Agric. Sci.*, vi, 4, December, 1914.

of Divers that sulphuretted hydrogen precipitates sulphur from a pentasulphide solution "as freely as it would arsenic or antimony" is quoted, and the assumption is made that sulphur is not freely precipitated from lime-sulphur solutions. This is quite erroneous, however, and a simple trial shows that in *dilute solution* sulphur is precipitated with extraordinary readiness. As already mentioned, the reaction is reversible, and no precipitation occurs until a certain relative concentration of hydrogen sulphide to calcium pentasulphide is reached. If Ramsay actually made the trial, it is clear that he must have worked with lime-sulphur solution in too high a concentration. In any case, such an argument used against the presence of pentasulphide would probably apply with equal force to the disulphide which Ramsay assumes to be present, since it is tolerably certain that the reaction with sulphuretted hydrogen is common to all polysulphides.

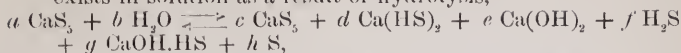
The argument that pentasulphide is decomposed by boiling its solution, and therefore cannot be present, is also invalid, since such decomposition is exceedingly slow and conditioned by the escape of traces of sulphuretted hydrogen produced by simple hydrolysis.

Ramsay also states that the disulphide is stable in water and assumes that the pentasulphide is not, but so far as we have been able to find out there is no evidence for assuming that one is more stable than the other. Indeed, it appears highly doubtful if a simple solution of the disulphide has ever been prepared at all, although, of course, it is a very easy matter to fake a solution of free hydroxide and higher polysulphide (dilute) in such proportions that the lime-sulphur ratio corresponds to a disulphide. The statement made in Thorpe's *Dictionary of Chemistry* (new edition, p. 610) that the disulphide may be crystallized out from the hot filtrate obtained by boiling sulphur with milk of lime, appears to be definitely wrong. We have ourselves attempted this mode of preparation, but have found that the passing of calcium hydroxide into solution is strictly regulated by the amount of sulphur used, in such a way that the proportions taken up correspond to higher rather than lower polysulphides, and a mixture of the highly soluble tetrasulphide and pentasulphide is usually obtained. Whenever crystals are obtained at all they are those of oxypolysulphides and not of the simple disulphide.

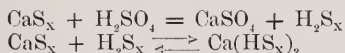
In regard to the contention that hydroxyhydrosulphide is present, little need be said except that there is no evidence in favour of it. This compound, equivalent to the monosulphide, can at most only be present in traces. The behaviour of a dilute lime-sulphur solution on titrating to acid with phenol phthalein as indicator, negatives the presence of any considerable amount of it, and the extraordinary ease with which, even in exceedingly dilute solution, it takes up sulphur to form higher polysulphide, renders it an almost impossible constituent in a solution prepared by boiling with excess of sulphur. Incidentally, also, Divers himself considers that hydroxyhydrosulphide does not exist in solution as such, but only as a mixture of hydroxide and hydrosulphide. The point is of no real significance, however, since it would be very difficult to determine the dissociation equilibrium of monosulphide in solution:—



Hydroxyhydrosulphide, or hydrosulphide and free lime, of course exists in solution as a result of hydrolysis,



but there is no evidence that any monosulphur derivative exists in the free unbalanced state. Theoretically of course it might exist, and a solution with a ratio corresponding to the expression $\text{CaS}_{4.5}$ might consist of a mixture of tetrasulphide with pentasulphide, or of pentasulphide with any mixture of lower derivatives from the monosulphide upwards. Until a series of pure polysulphides is actually isolated, and the behaviour of mixtures studied, it would seem impossible to determine whether an unknown solution with a high calcium-sulphur ratio consists of a mixture of higher sulphides alone or of higher sulphides with lower members. With the analytical methods now available, it is possible to determine Ca and x in the expression CaS_x , but that is all. By reference to the known behaviour of the monosulphide towards acid titration with various indicators, and towards free sulphur, it is possible to determine whether any *large* quantity of this lowest derivative is present, but it is extremely difficult to gauge the distribution of x. The possible existence of homologues higher than the pentasulphide must also be considered, although until recently the general consensus of prejudice has been against any such assumption. Finally, the possibility of the existence, under certain conditions, of hydropolysulphides of the type $\text{Ca(HS}_x)_2$, analogous to the accepted hydrosulphide Ca(HS)_2 , cannot be ignored, although the concordant titrations to acid and to zinc negative this in general. If the existence either of hydropolysulphides or of normal derivatives higher than the pentasulphide be admitted, the observed occurrence in solution of atomic calcium-sulphur ratios above 1 : 5 can be readily explained. As already mentioned, we have ourselves noted a ratio 1 : 5.4 in *freshly made* lime-sulphur solutions, and a ratio of over 1 : 5.1 appears to be quite common in moderately strong solutions which have been stored for a considerable time. We have also noted that even in a solution of ratio 1 : 5, the addition of acid or of iodine (during process of titration) does not cause an immediate permanent precipitation of sulphur, but that sulphur precipitated in areas of local excess redissolves up to a point well above the pentasulphide. We were inclined to explain this upon the assumption of a reaction of the type—



assuming that only a limited formation of hydropolysulphide could occur owing to the instability and ready dissociation of hydrogen polysulphides—



The analogy for such a reaction of course exists in the case where $x = 1$; where on addition of H_2S to the monosulphide a neutral point to phenol phthalein is reached at exactly the stage represented by Ca(HS)_2 .

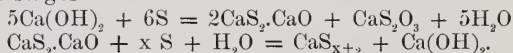
As against this suggestion there is the possibility that the redissolved sulphur is held in physical solution in combined form, but the behaviour noted in one case in which a solution originally containing sulphur in the ratio $\text{Ca:S}::1:5.4$ and had *deposited* excess of sulphur on standing, still appeared capable of redissolving small

quantities of sulphur precipitated by acid, renders explanation very difficult.

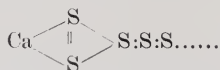
When Auld's paper appeared we found that he had carried the observation of the behaviour towards acid still further, and had considered the re-solution of sulphur at varying temperatures. Titrating at 90° C., he obtained evidence of a ratio Ca : S : : 1 : 6.1, suggesting the existence of a heptasulphide. In this connection it is interesting to note that Rule and Thomas⁵ have recently made a similar suggestion for the sodium-sulphur derivatives, and in the course of an investigation in which they definitely establish sodium disulphide and sodium tetrasulphide as well-defined crystalline compounds, they indicate the existence in *alcoholic solution* of members higher than sodium pentasulphide.

So far as we had been able to find out, no normal polysulphide of calcium has been isolated in the pure state, although from Auld's paper we note that Van Slyke, Bosworth, and Hedges claim to have obtained crystals corresponding to the tetrasulphide and pentasulphide from a concentrated lime-sulphur wash. Up to the time of writing we have not been able to procure the original paper of Van Slyke, Bosworth, and Hedges, or to discover exactly how detailed an examination of the crystals analysed by them was actually made. The isolation of a solid tetrasulphide (or oxytetrasulphide), however, appears to us quite feasible.

Auld, although quoting the claim of the American workers, nevertheless appears to attach little or no significance to it, concentrating rather upon the fact that any of his own attempts to prepare crystalline polysulphides resulted in the formation of Herschell's crystals (to which the formula $\text{CaS}_2 \cdot \text{CaO} \cdot 7\text{H}_2\text{O}$ is assigned), and that the extraction of dried polysulphide material with carbon disulphide gave a product with a ratio monosulphur equivalent : total sulphide sulphur : : 1 : 1.51, concludes that the oxydisulphide $\text{CaS}_2 \cdot \text{CaO}$ is the stable primary derivative from which the higher sulphides are built up. He explains the reactions between free sulphur and lime as taking place in two stages—



He then suggests that the polysulphides of calcium possess the constitution



the atoms of sulphur in the chain becoming progressively more loosely attached, and (p. 484) considers that as the chain lengthens the last added are so loosely attached as to behave practically as *sulphur in solution*.

We do not wish to cavil at this view of the constitution of the polysulphides, or of the primary reaction between lime and sulphur, but at the same time we think that the evidence upon which it is based is open to serious objection. Our own results dealing with the behaviour of polysulphides (either of calcium or of sodium) in aqueous solution had led us to the conclusion that the sulphur in the higher derivatives is very firmly attached, and it is quite certain that the atoms above the two directly attached to calcium in Auld's formula

⁵ *J.C.S.*, January, 1915.

show no sign of behaving like "sulphur in solution." The experiments carried out in connection with the work on the sulphur dips showed that sulphur combined as pentasulphide was much more stable than *powdered roll sulphur* towards free base. No matter what proportions of lime and sulphur, or caustic soda and sulphur, were boiled together, higher polysulphides rather than lower polysulphides were invariably formed, while the excess of base remained free in solution. The following data illustrate this point:—

- A. Pure slaked lime, 100 grammes (CaO, 72 grammes), and sulphur, 40 grammes, i.e. a proportion of sulphur less than that required for the formation of monosulphide, were boiled together with 250 c.c. of water for forty minutes with frequent shaking. Practically all the sulphur dissolved, and a deep orange-red solution was obtained. While still hot a portion was filtered from the large excess of unused lime and stored in a small stoppered bottle (full).
- B. Caustic soda, 5 grammes, and sulphur, 1.8 gramme (i.e. approximately in the proportions required for the monosulphide), were boiled together with 50 c.c. of water. In ten minutes practically all the sulphur was dissolved, giving a solution whose deep colour at once indicated the presence of polysulphide. The slight residue of coarser undissolved sulphur was filtered off.

Aliquots of these two clear solutions, suitably diluted to concentration convenient for titration with $N/_{10}$ reagent, gave the following data:—

- A. 20 c.c. diluted solution + 50 c.c. of water
 titrated to $N/_{10}$ ammoniacal zinc = 45 c.c.
 Filtered and titrated to $N/_{10}$ I_2 for thio-
 sulphate = 13.2 c.c.
 Sulphate and sulphite: trace.
 20 c.c. for total sulphur = 0.418 gm. S.
 20 c.c. titrated to $N/_{10}$ acid and methyl
 orange... .. = 45.3 c.c.
 20 c.c. precipitated with oxalate for CaO and
 titrated to $N/_{10}$ $K_2Mn_2O_8$ = 71.9 c.c.
 45 c.c. $\times 0.0016 = 0.072$ gramme mono-
 sulphur equivalent.
 13.2 c.c. $\times 0.0064 = 0.084$ gramme thio-
 sulphate sulphur.

Subtracting thiosulphate sulphur from total sulphur and ignoring the trace of sulphate, we have total sulphide sulphur 0.334 gramme, which, divided by the monosulphur equivalent 0.072, gives a quotient 4.64, corresponding to the expression $CaS_{4.64}$, or a mixture of tetrasulphide and pentasulphide.

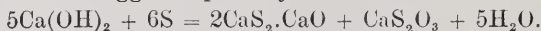
At the same time, the approximate agreement between the acid titration and the zinc titration, together with the close agreement between the calcium oxide as calculated ($45 + 13.2 \times 2 = 71.4$ c.c.) and as actually found (71.9 c.c.), indicates the absence of any considerable amount of free lime or oxypolysulphide. Any free lime present in the hot solution may possibly have deposited as oxypolysulphide during filtration and have been retained in the residue of unused lime.

B. 20 c.c. diluted solution + 50 c.c. water +
 10 c.c. 10 per cent. NH_4Cl to remove
 free NaOH , titrated to $\text{N}/_{10}$ ammon. zinc = 10.0 c.c.
 Filtered and titrated to $\text{N}/_{10}$ I_2 for thio-
 sulphate = 3.3 c.c.
 Sulphate and sulphite: trace.
 20 c.c. for total sulphur... .. = 0.083 gm. S.
 10 c.c. $\times 0.0016 = 0.016$ gramme monosulphur equivalent.
 3.3 c.c. $\times 0.0064 = 0.021$ gramme thiosulphate sulphur.
 Subtracting the thiosulphate sulphur from the total sulphur,
 we have total sulphide sulphur 0.062 gramme, which, divided
 by monosulphur equivalent 0.016, gives a quotient 3.88,
 corresponding to the expression $\text{Na}_2\text{S}_{3.88}$, or approximately
 the tetrasulphide.
 20 c.c. aliquot of diluted B was now titrated to $\text{N}/_{10}$ acid,
 using methyl orange as indicator, and found to register 49 c.c.
 This indicates the presence of $49 - 10 = 39$ c.c. equivalent of
 free caustic soda, or approximately four times that actually
 combined as polysulphide. A rough titration for alkalinity
 (c.c. $\text{N}/_{10}$ acid required to neutralize before titrating for thio-
 sulphate) on the filtrate from the zinc precipitate, compared
 with a blank titration for alkalinity (original free ammonia)
 on the ammoniacal zinc reagent used, indicated a similar
 amount of free base in solution B.

From these data, A and B, it is quite apparent that higher poly-
 sulphides are formed in preference to lower polysulphides even on
 boiling with excess of free base. In case A the lime was taken in huge
 excess of the sulphur, but the greater part remained unused, and the
 resulting solution corresponded to a mixture of tetrasulphide with
 pentasulphide. In case B the tetrasulphide is suggested as the most
 stable derivative in alkaline medium.

According to the supposition of Auld, calcium oxydisulphide
 $\text{CaS}_2 \cdot \text{CaO}$ should have been formed in A, giving a ratio monosulphur
 equivalent : sulphide sulphur : 1 : 2, instead of 1 : 4.6 as actually
 found, and showing an equivalent of free lime equal to that of the
 lime in combination as sulphide—i.e. showing 10 c.c. instead of 0.3 c.c.
 actually found.

This does not suggest a primary reaction—



If this reaction had taken place there was no reason why the second
 reaction



should have followed, since excess of lime, and not excess of sulphur,
 was used. On the contrary $\text{CaS}_2 \cdot x\text{CaO}$ (p. 483) should have been
 formed.

According to Auld's view that the sulphur in a polysulphide
 solution, above that contained in the disulphide, is in a loose state of
 combination and behaves as *free sulphur*, pentasulphide should be
 reduced to disulphide (or oxydisulphide) by boiling with excess of
 lime.



In this case free lime should be taken up to combine with "loosely
 attached" sulphur, and by boiling with excess of hydroxide the

monosulphur equivalent should be *doubled*. A few tests were carried out in which pentasulphide was boiled with large excess of lime water, and although the solubility factor (of lime) was thus excluded, it was found that very little increase in monosulphur equivalent took place and that the pentasulphide was only slowly reduced. The following simple experiments illustrate this point, and show that "polysulphide sulphur" is firmly attached and does not behave like free sulphur:—

- (a) 20 c.c. diluted lime-sulphur concentrate + 200 c.c. water + 10 c.c. 10 per cent. NH_4Cl , titrated to ammoniacal zinc gave a reading of 32.3 c.c. for monosulphur equivalent (0.0517 gramme S.) and 1.8 c.c. $\text{N}/_{10} \text{I}_2$ for thiosulphate (0.0115 gramme S.) in the filtrate. The total sulphur in 20 c.c. was determined as 0.270 gramme, and the ratio of monosulphur equivalent to total sulphide sulphur in the solution was therefore 5.0, corresponding exactly to the pentasulphide.
- (b) To 20 c.c. of the same solution was added 200 c.c. of clear lime water, equivalent to 95 c.c. $\text{N}/_{10} \text{CaO}$, and the whole boiled for 20 minutes and then rapidly cooled. 10 c.c. of 10 per cent. NH_4Cl was now added to replace free lime with ammonia (which in moderate amount does not affect the zinc titration), and the solution titrated to zinc. A monosulphur equivalent of 38.3 c.c. was registered, and a thiosulphate equivalent of 3.9 c.c. $\text{N}/_{10} \text{I}_2$. During boiling, an amount of lime (less soluble in hot than in cold solution) was *precipitated*, which, when filtered off (in a duplicate test) on asbestos, rapidly washed with a few successive cubic centimetres of hot water and titrated to $\text{N}/_{10}$ acid and phenol phthalein, registered 47 c.c. The absence of sulphide, sulphite, and sulphate was established in this precipitate by testing with iodine, and with barium chloride after solution in acid. It therefore consisted of pure hydroxide. The filtrate from the precipitated lime gave a total acid titration to methyl orange of 74.8 c.c.

Hence:—

Added lime	= 95 c.c. $\text{N}/_{10}$
Converted into sulphide, 38.3 c.c.—32.3			
c.c.	= 6 c.c.
Converted into thiosulphate, 3.9 c.c.—1.84			
c.c.	= 2.1 × 2
			= 4.2 c.c.

Remaining free in solution, 74.8 c.c.—

38.3 c.c.	= 36.5 c.c.
Precipitated out as free lime	= 47 c.c.
Lime accounted for = 93.7 c.c., leaving			
1.3 c.c. unaccounted for as sulphate,			
carbonate, and "error."			

Monosulphur equivalent = $38.3 \times 0.0016 = 0.0613$ grm. S.

Thiosulphate sulphur = $3.9 \times 0.0064 = 0.025$ grm. S.

Total sulphide sulphur = $0.270 - 0.025 = 0.245$ grm. S.

Hence ratio $0.245 : 0.0613 = 1 : 4$, corresponding exactly to the tetrasulphide CaS_4 .

(N.B.—A duplicate trial gave $\text{CaS}_{1.25}$.)

- (c) To 20 c.c. of the same solution was now added the same amount of lime water (200 c.c.) and large excess of free sulphur (a few grammes of aqueous paste). This mixture was then boiled for the same length of time (twenty minutes) as in the last test, filtered, washed from excess sulphur and cooled. On titration with ammoniacal zinc, 90.3 c.c. were now required for monosulphur equivalent and 19.6 c.c. $N/10$ I_2 were required for reaction with thiosulphate after filtration and neutralization.

Hence:—

Added lime	= 95 c.c. $N/10$
Converted into polysulphide, 90.3 c.c.—	
32.3 c.c.	= 58 c.c.
Converted into thiosulphate, 19.6 c.c.—	
1.8 c.c.	= 17.8×2
	= 35.6 c.c.

Total 93.6 c.c. accounted for, leaving
1.4 c.c. in other form or as "error."

From (b) it is evident that higher polysulphides of calcium are stable in dilute solution even on boiling with excess of lime, and that the pentasulphide is only slowly reduced *towards* the tetrasulphide. Added free lime is partly precipitated, partly remains in solution, and, to a slight extent, is used in reducing the ratio of 1 : 5 down to 1 : 4 or 1 : 4.25.

From (c), under the same conditions, it is seen that in presence of *added free sulphur*, the added lime is practically quantitatively taken up to form further polysulphide—pentasulphide, since a little free sulphur separated out on cooling the filtered liquid.

Here we have a direct comparison between the behaviour of "free sulphur" and of "polysulphide sulphur." Obviously the polysulphide sulphur in a lime-sulphur preparation is not so loosely attached as to behave like "free sulphur in solution," but is, on the contrary, astonishingly firmly combined.

In exactly the same way it may be shown that the sulphur in sodium pentasulphide is firmly combined. After boiling an $N/10$ solution of sodium pentasulphide for half an hour with sufficient excess of free caustic soda to theoretically reduce to the monosulphide, the actual reduction only proceeded as far as the expression $Na_2S_{4.2}$, i.e. towards the tetrasulphide. The added caustic soda remained as free hydroxide and could be almost quantitatively established as its equivalent of free ammonia in the filtrate from the ammoniacal zinc precipitate. As a check upon the zinc method, a determination was carried out with acid copper sulphate in hot solution, in which the copper precipitated as sulphide was determined. Although accurate results could not be obtained owing to the interfering presence of thiosulphate in the polysulphide solution, the presence of the tetrasulphide was confirmed.

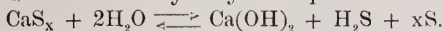
By boiling with free alkali, therefore, *even in very dilute solution* ($N/100$), the value of x in the expression CaS_x or Na_2S_x is only slowly lowered. With the analytical methods at disposal it is impossible to say what particular mixture of lower sulphides is actually formed. The general evidence, however, points rather to the tetrasulphide as the most stable derivative. We regard our data as effectually disposing of the contentions both of Auld and of Ramsay in regard to "loosely attached sulphur" and "sulphur in solution,"

The data from which Auld drew his conclusion that the primary stable derivative is the oxydisulphide $\text{CaS}_2 \cdot \text{CaO}$ remain to be explained away.

Three points have to be considered—

- (a) the behaviour of polysulphide sulphur in solution towards sulphur solvents;
- (b) the extraction of sulphur from desiccated lime-sulphur preparations;
- (c) the crystallization of oxypolysulphides from lime-sulphur solutions prepared by the use of excess of lime over sulphur.

The first point may be dismissed very shortly, since we do not think that the data given by Auld allow of any interpretation other than progressive removal of hydrolytic sulphur—



In the process of extraction, both sulphuretted hydrogen and sulphur may be slowly removed, so shifting the equilibrium forward and finally effecting complete extraction of all the sulphur. In point of fact, a removal of 95.7 per cent. in 47 hours (p. 490) is actually established. The remaining 4.3 per cent. may represent traces of sulphide very difficult to extract in presence of free base, or possibly correspond to slight conversion of sulphur into non-sulphide form. The author, however, considers that the *rate* of extraction probably indicates a slower removal of sulphur at the time when the atomic calcium-sulphur ratio is in the neighbourhood of 1 : 2, and considers this as evidence for the presence of a more stable disulphide complex formed in the process of extraction.

It is true that very little emphasis is laid upon this evidence, but we hardly think that it can be used as evidence at all. If we rearrange Auld's data so as to show extraction *per hour*, we have:—

Time of Extraction.	Sulphur Removed.	Sulphur Removed per Hour.
First 7 hours	0.0631 grm.	0.00902 grm.
Second 6 hours	0.0567 „	0.00945 „
Third 8 hours... ..	0.0485 „	0.00606 „
Fourth 6 hours... ..	0.0276 „	0.00490 „
Fifth 5.7 hours	0.0167 „	0.00293 „
Sixth 8.5 hours	0.0167 „	0.00185 „
Seventh 6 hours	0.0121 „	0.00201 „

Considering the normal irregularities to be expected in liquid extraction with any ordinary apparatus and the fact that the composition (free base) of the solution is subject to progressive change, we do not think that these figures show anything more than slow removal of sulphur, diminishing more or less steadily as extraction proceeds. The irregularities of the “rate curve” are hardly characteristic enough to justify the assumption that an intermediate, relatively stable, oxydisulphide is present at any time.

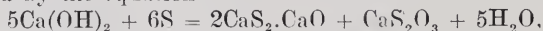
(b) On mixing desiccated polysulphide material with silver sand and extracting with carbon disulphide. Auld obtained a residue which, on dissolving in water, gave a ratio monosulphur equivalent: total sulphide sulphur : : 1 : 1.51. Although this does not correspond to the ratio required for the oxydisulphide (1 : 2), it is (p. 495) taken as evidence of the probable existence of a stable disulphide complex. Unfortunately this ratio, 1 : 1.51, is invalidated by the fact that the analysis upon which it is based was carried out by the method of

Harris (footnote, p. 491). This method, as we have indicated earlier, is invalid in presence of free lime, and the dried extracted powder may well have been simply a mixture of free lime with any higher polysulphide or oxypolysulphide.

(c) In the attempted preparation of the disulphide, Auld boiled 56 grammes of quicklime and 64 grammes of sulphur in 1 litre of water and filtered hot. On analysis the liquid gave a ratio, monosulphur equivalent : total sulphide sulphur, of 1 : 5 in one case and 1 : 4.8 in another. Instead of accepting this as evidence of the formation of a stable pentasulphide, the author goes on to consider the fact that on standing, Herschell's crystals, with a ratio of 1 : 2, separated out. This fact he uses as evidence for the existence of a stable intermediate disulphide complex. Without attempting to dispute the obvious existence of such a complex, we cannot see that it is of any importance in so far as the main reaction is concerned. In using a litre of water for boiling the lime-sulphur mixture, scope was afforded for the solution of an appreciable amount of free lime. The subsequent separation of this in the form of Herschell's crystals may merely mean that the oxydisulphide represents the *least soluble* oxysulphide complex, but not necessarily the *most stable*. It is certainly very thin evidence with which to support any conclusions concerning the *primary* reaction between sulphur and lime in aqueous solution. The formation of oxysulphides may just as easily be secondary and the crystallizing out of these be conditioned simply by their solubility. It is interesting to note (p. 493) that data corresponding to a disulphide complex were only obtained with the first crops of Herschell's crystals as prepared by Auld's modification of Geuther's method. Although no analyses of later crops are actually given, Auld states that they appeared to differ somewhat in composition.

The astonishing thing in the particular experiment under consideration is that a disulphide complex separated out from a solution with a ratio monosulphur equivalent : total sulphide sulphur : 1:5. Such a separation of $\text{CaS}_2 \cdot \text{CaO}$ suggests the existence of a stable derivative higher than the pentasulphide in the cold mother liquor.

Although the calcium equivalent of the solution is not stated, we have ourselves found that the evidence, in solutions prepared in the same way, is in favour of the presence of pentasulphide and free lime rather than of oxypolysulphides. On cooling a hot solution prepared by using excess of lime over sulphur, the amount of oxypolysulphide which crystallizes out is very small and appears to be determined by the bulk of water used in boiling, i.e. by the extent to which free lime has been allowed to go into simple solution. In any case if, as Auld thinks, the primary reaction between sulphur and lime is to be represented by the equation



and if the oxydisulphide is more stable than the pentasulphide, then all the sulphur used should have appeared in disulphide proportions, either in solution or in insoluble form as sediment. Instead of this, Auld's own data show sulphur in solution in form corresponding to the pentasulphide (1 : 5 ratio).

We therefore consider that the reaction between lime and sulphur in aqueous solution is still only capable of being written in final empirical form,



and that the intermediate steps in the reaction are as obscure as ever.

It appears at least possible that the series of derivatives CaS_x are all stable once they are formed, but that in presence of excess of sulphur the tendency is always for the formation of higher polysulphides. The monosulphide, for example, is perfectly stable, yet in presence of free sulphur at once passes into polysulphide—the pentasulphide for preference.

In this connection the behaviour of sulphur towards sodium hydrosulphide in alcoholic solution, as studied by Rule and Thomas (*l.c.*), is of great interest. These authors found that the tendency was towards formation of tetrasulphide, no matter how little sulphur was used, and that this derivative could not be reduced by excess of sodium ethoxide, but only by sodium itself (nascent hydrogen).

It would be interesting to know exactly how the disulphide Na_2S_2 , as prepared by Rule and Thomas, behaves towards free sulphur and towards indicators such as phenol phthalein, and to know whether mixtures of sodium monosulphide, disulphide, and tetrasulphide (all now preparable in the pure state) can be discriminated in solution by any of the known analytical methods.

We regret that limitations of time prevent any decent study of the calcium-sulphur derivatives in their behaviour towards various reducing agents or the detailed investigation of lime-sulphur solutions in which free lime is absent but which show a ratio, monosulphur equivalent:total sulphide sulphur, below 1:5. Does the solution then consist of mixture of pentasulphide with monosulphide or are intermediate polysulphides present?

SUMMARY.

1. A general discussion of the validity of the iodine titration method of Harris for the analysis of polysulphide mixtures is offered, and preference is given to the older methods involving the use of ammoniacal zinc chloride. In the presence either of free lime or free sulphuretted hydrogen, the iodine titration method countenanced for the analysis of lime-sulphur solutions, by the American Association of Official Agricultural Chemists, is altogether invalid, but by combining the ammoniacal zinc method with simple titration to standard acid, reasonably accurate results may be obtained, whatever be the composition of the polysulphide solution.

2. The recent views of Ramsay and of Auld in regard to the nature of lime-sulphur solutions and to the reaction involved in their manufacture are discussed and criticized as invalid. Apart from the presence of thiosulphate, the dominant constituent is calcium pentasulphide, and "polysulphide sulphur" is regarded as firmly combined. Even if huge excess of free lime over sulphur be used in preparation, the tendency always is towards formation of higher rather than lower polysulphides, the higher derivatives being stable even on boiling with free base.

The possibility of the existence of derivatives above the pentasulphide is also discussed.

Arsenical Dip-Tester.

BY

HENRY H. GREEN,

Biochemist, Division of Veterinary Research.

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A ROUGH field test to ascertain the active arsenical content of cattle dips is very necessary in any area where systematic dipping against ticks is practised. The necessity for keeping the volume of liquid at working level in a constantly used tank, for correcting the concentration after dilution by rain-water, or after alteration by evaporation or oxidation, renders the periodic testing of dips almost indispensable.

To meet the need for an apparatus capable of simple and rapid manipulation in field practice several instruments have been devised. The firm of Wm. Cooper & Nephews have recently informed us that they have constructed a tester for use with their own proprietary cattle dip, but although this instrument is apparently upon the American market* it is not available in South Africa. We have also come across a reference to the use of an arsenical dip-tester by the United States Bureau of Animal Industry, but so far we have been unable to procure the original description of the instrument.† In this country the only apparatus on the market is sold as a proprietary article by Messrs. Henwood, Soutter & Co., under the name of *Isometer*, but the cost of the apparatus itself—and more particularly of the materials used in carrying out the test—is so high that the present writer was asked if a cheaper substitute could not be devised, so as to enable the farmer to test his dip more cheaply than he can to-day.

The following brief description of alternative instruments represents the reply made to that question, now published on instructions received from the Director of Veterinary Research.

The cattle dips chiefly used in this country consist of a simple solution of commercial arsenite of soda, with or without the addition of soft soap and paraffin. In the so-called "laboratory dips," made up upon the Watkins-Pitchford formulae, the use of soft soap and paraffin is recommended, but at the present time simple aqueous solutions of arsenite are often used‡—one lb. of 80 per cent. arsenite of soda (the usual commercial grade) per 100 gallons of water, for immersion at three-day interval; 2 lb. for the five to seven day interval; and 3 lb. for the fourteen-day dipping.

* In the United States it appears to be obligatory for every manufacturer of an approved proprietary dip to supply a tester with which to control the tanks where that particular proprietary dip is in use.

† Since obtained—see appendix to this article.

‡ W. F. Cooper (*Journ. Agric. Sc.*, vol. vii, part 2, and *Parasitology*, vol. 8, No. 2) is a strong advocate of the use of soap in the dipping fluid, on the grounds that the "wetting power" is thereby increased, and any given concentration of arsenite rendered more effective.

In testing the ordinary cattle dip it is therefore only necessary to determine the amount of arsenite present. This may then be stated as percentage of arsenious oxide in accordance with analytical convention, or, in a fashion more intelligible to the farmer, as "ounces of commercial arsenite of soda per 100 gallons of dipping fluid."

The basic process which at once suggests itself as suitable for a field test is of course the familiar textbook reaction taking place between arsenious compounds and iodine in alkaline medium:—



In the time-honoured laboratory process of estimation, a solution of iodine of any standard strength is run into any measured aliquot of arsenious solution rendered potentially alkaline with sodium bicarbonate, and with or without the use of starch as indicator. The type of laboratory apparatus used may vary with the whim of the analyst and the degree of accuracy required, but in each case the relative quantities of standard iodine and arsenite solution which react with one another are determined, and from this the content of arsenious oxide is calculated.

This familiar iodine reaction is obviously the basis of Cooper's tester, of the Isometer, and presumably also of the U.S.A. Bureau instrument.

The only excuse for presenting a fourth apparatus lies in the hope that some firm will undertake to put an article on the South African market, together with a regular supply of materials required for the test, at a cost much below that of the existing Isometer. The Isometer as such is patented but, since it is impossible to patent a time-honoured analytical process, an instrument along the lines indicated in this note should not only be immune from accusation of infringement, but be itself incapable of patent.

The simplest type of rough apparatus which could be used would obviously consist of an ordinary measuring cylinder. A portion of the dip to be tested could simply be poured in, and the quantity taken read off. After addition of a little sodium bicarbonate, a standard solution of iodine could be poured in slowly (with shaking of the cylinder) to an end-point shown by starch paper, and the quantity used then read off as the second level in the cylinder. From these data and the known arsenious equivalent of the iodine, the arsenite content of the dip could be calculated. Obviously, however, the calculation itself may be embodied either in the iodine solution or in the system of graduation of the cylinder. If the iodine solutions be made of strength equivalent to the dips to be tested the whole calculation can then be embodied in a simple system of graduation.

For convenience in handling, a hard glass test-tube is preferable to a cylinder, and the graduations may therefore be most conveniently inscribed upon tubes.

Strong glass test-tubes of 30 c.c. capacity and graduated at intervals of $\frac{1}{2}$ c.c. from top to bottom are already marketed at 8½d each, retail (Warmbrunn, Qinitz & Co., Berlin, Catalogue No. 4023). These are supplied with and without lips, and two of these, one unlippped for the dip and one lippped for the iodine solution, constitute all the "instrument" required—at a cost of 1s. 5d. If the unlippped tube were filled to the 10 c.c. mark with dip, a "pinch" (as much as would lie on a threepenny bit) of sodium bicarbonate

added, and iodine solution, 1 c.c. = 1 mg. of sodium arsenite, added to end-point as shown by starch paper, each c.c. above 10 (i.e. each c.c. of iodine added) would indicate .01 per cent. of sodium arsenite or $\frac{1}{10}$ of a lb. per 100 gallons. Thus, if after adding iodine, the end-point occurred at 20 c.c., the amount of iodine added would be 10 c.c., and .1 per cent. of sodium arsenite (.08 per cent. As_2O_3), or 1 lb. per 100 gallons (standard three-day strength), would be indicated. If the end-point occurred at 18 c.c., then 8 c.c. of iodine has been added, and the presence of .08 per cent. of sodium arsenite, or $\frac{8}{10}$ lb. per 100 gallons would be indicated. $\frac{8}{10}$ lb. is approximately 13 ozs., and if 16 ozs. constituted standard dipping strength it would obviously require the addition of 3 ozs. of arsenite of soda per 100 gallons of dip to restore to required strength. In the same way if the reading stood at 22 c.c., then 12 c.c. of iodine has been added and the dip would contain $\frac{12}{10}$ lb. of sodium arsenite per 100 gallons (.12 per cent. of arsenite, or .096 per cent. As_2O_3). To restore to standard strength of 1 lb. of arsenite, every 100 gallons of dip would have to be made up to $100 \times \frac{10}{12} = 120$ gallons, i.e. 20 gallons of water would have to be added to each 100 gallons of tank fluid.

The range of a tube of 30 c.c. would be 2 lb. of arsenite per 100 gallons (standard five-day strength). A second iodine solution would therefore have to be provided for concentrations above this, but the calculation required to determine the data necessary for correction of any dip to any required standard would be equally simple and could be carried out mentally. If desired, however, a table could easily be constructed showing all the data required, simple reference to which would avoid all calculation.

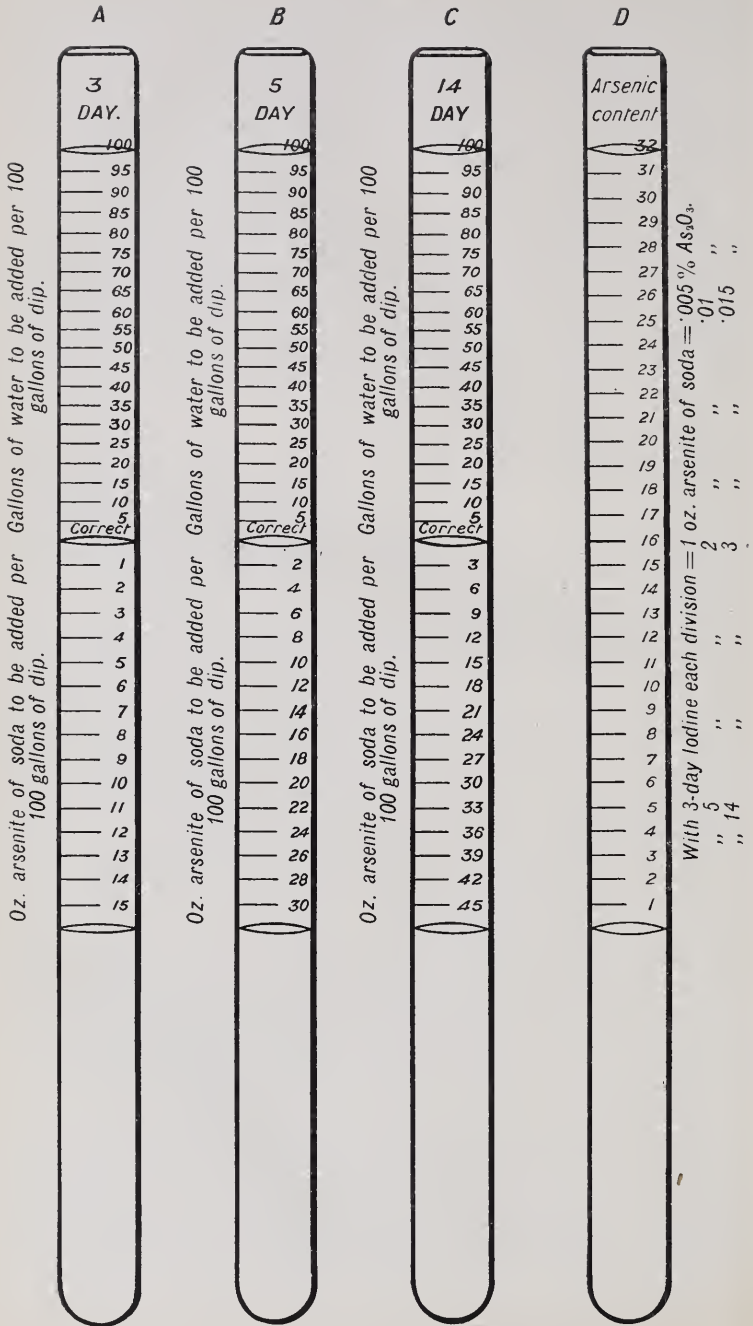
The cost of such an apparatus would be trifling—two tubes at 8½d. each. If the iodine were poured in from the stock bottle only one tube would be required, and the primitive “dip-tester” would then cost 8½d.—say 1s. in South Africa. It might be worth while for some South African firm to stock these tubes. The cost of carrying out each test would be less than 1d.—e.g. for fifty tests of three-day dip, there would be required: one book of starch papers, 2d.; $\frac{1}{4}$ lb. of sodium bicarbonate, 1½d. (or in corked bottle, 3d.); a half-litre bottle of a solution of iodine in potassium iodide containing .205 per cent. iodine ($\pm .001$ would not matter) at about 2s. 6d.

Naturally, however, it is worth while constructing the test-tubes with more convenient graduations, and putting a compact box of materials together for special use.

If a test-tube be divided into equal capacities (say three) and the lowest of these filled with standard dip, the end-point will obviously occur when the equivalent iodine solution has been poured in up to the second dividing mark. If the dip is above standard the end-point will occur above this mark, and if below standard will occur below. All that is necessary to determine the strength of an unknown dip is to graduate the tube above and below the second mark.

Since dips are made up in terms of lb. of arsenite of soda per 100 gallons of fluid, the most convenient system of graduation consists in dividing the tube above and below the second mark into capacities each *one-sixteenth* of the three primary divisions. The graduations will then read directly in *ounces of arsenite of soda per 100 gallons of dip*. Since it happens that commercial arsenite is sold on a basis of 80 per cent. As_2O_3 , the same divisions will simultaneously

Figure 1.



correspond to a simple decimal percentage of arsenious oxide—0.005 per cent., 0.01 per cent., or 0.015 per cent., according to whether the iodine solution used is equivalent to 1 lb., 2 lb., or 3 lb. of arsenite per 100 gallons, i.e. according to whether the iodine corresponds to three-day, five-day, or fourteen-day dip.

Such a test-tube, divided into three equal capacities, with the upper two sub-divided into sixteenths, as diagrammatically shown in D, fig. 1, therefore suffices to determine directly, and without calculation other than simple mental multiplication, either the percentage of arsenious oxide or the number of ounces of arsenite of soda present in 100 gallons of solution.

The farmer, however, wishes to know not so much the quantity of arsenite actually present as the quantity of arsenite, or of water, to be added to bring the dip to standard strength. A simple calculation is then necessary, although this may be avoided by reference to tables constructed for the dips in use. Table I, printed here, gives all the necessary data required for correcting the three standard dips. An example of the use of this table, and of the calculation required when a dip of arbitrary strength is to be corrected, will be given presently.

Wherever standard dips are in use it is a simple matter to embody such calculation on the tube itself. For the three standard dips in common use in South Africa it is therefore advisable to provide three tubes—as indicated in A, B, and C, fig. 1. Such tubes are divided on their uppermost third into twenty equal graduations, and on their middle third into sixteen. If the upper twenty divisions be numbered consecutively upwards—5, 10, 15 . . . 100—we have a direct reading, at convenient intervals of five, of the *number of gallons of water which must be added to each 100 gallons of tank dip to restore to standard strength*. Below the second mark the sixteenths are numbered consecutively downwards. For each tube the actual graduation marks are the same, but the numbers proceed at intervals of one in the three-day tube, of two on the five-day tube, and of three on the fourteen-day tube. The figures then show the *number of ounces of arsenite of soda to be added per 100 gallons of tank fluid to restore to standard strength*.

As already indicated, an equivalent solution of iodine is required for each of the three standard dips, to be used without calculation with tubes A, B, and C, fig. 1, or with tube D by calculation or in conjunction with Table I.

The concentrations of these three solutions are:—

Three-day—1 lb. arsenite per 100 gallons = .08 per cent. As_2O_3 = $.08 \times \frac{12.692}{4.948}$ = .205 per cent. iodine.

Five-day—2 lb. arsenite per 100 gallons .410 per cent. iodine.

Fourteen-day—3 lb. arsenite per 100 gallons .615 „ „

These solutions are most easily made up by dissolving 41 grammes of pure iodine and 100 grammes of pure potassium iodide per litre of water, and using this as stock—50 c.c., 100 c.c., or 150 c.c. being diluted to 1000 c.c. with water, according as three-day, five-day, or fourteen-day solution is required. Standardization in bulk, against thiosulphate or arsenious oxide, may be made either on the stock fluid or on the diluted solutions. So high a degree of accuracy is naturally not required for field work as for laboratory practice, but at the same time it is as easy to turn out solutions exactly correct as it is to make them approximate. The solutions could then be sold retail in properly

labelled 8-oz. glass-stoppered bottles, either N.M.F.S. or Winchester pattern.

The testing tubes can of course be of any dimensions at all, provided that they are graduated into equal capacities in regard to the three primary divisions. A size approximately 25 cm. \times 1.5 cm. would perhaps be most convenient, and, if of uniform bore, intermediate graduations could be scratched off by linear sub-divisions as in the case of cheap quality burettes. With this size of tube each major third would have a capacity of approximately 10 c.c. The three major divisions should be indicated by circular scratches carried round the tubes, to distinguish them from the shorter intermediate graduations. The tubes should be of stout glass, and either slightly constricted at the top, or evenly cut and neatly rounded off, so as to allow of closing the aperture with the thumb in shaking. The requisite information (as indicated in fig. 1) concerning each tube could be inscribed upon the back, behind the graduations, with the words three-day, five-day, fourteen-day, and "arsenic content," respectively, marked at the top.

In addition to the iodine solution, and the testing tubes, a small mixing bottle, a sheaf of starch papers, and a stock of bicarbonate, are necessary. The mixing vessel need simply consist of an ordinary 4-oz. wide-mouthed, moulded "kali" bottle (costing a penny), provided with a cork. For preference the bottle should be marked with a sand-blasted line, roughly indicating a capacity of 100 c.c. Such marking is not absolutely necessary, however, since these bottles hold a suitable quantity of fluid when about four-fifths full. The starch papers as usually sold in book form at twopence a book, are suitable. Since soap is a constituent of cattle dips made up upon the Watkins-Pitchford formulae, it is advisable to mix some salt of a metal capable of forming an insoluble soap, along with the sodium bicarbonate. Where soap is absent no harm is done. Magnesium sulphate serves this purpose and the "reaction powder" should therefore consist of an intimate mixture of sodium bicarbonate and magnesium sulphate. Roughly equal weights of each salt may be used. The mixture may either be put up in stock bottles with a small horn-spoon which, when filled level, holds from 2 to 3 grammes of the mixture, or it may (more conveniently) be put up in the form of "powders," each weighing 2-3 grammes, and turned out in cardboard boxes of two dozen.

The Test.—In carrying out the test the mixing bottle is filled to about four-fifths with the dip to be tested (or up to the sand-blasted line), and a level spoonful (or one powder) of bicarbonate mixture emptied in. The bottle is corked and shaken for about half a minute to dissolve the powder. Selecting the appropriate testing tube, the alkalinized dip is then poured in from the mixing bottle until the lower surface of the curved meniscus is on the lowest circular mark. If the mark is overshot in pouring in the dip a few drops may be allowed out by closing the tube with the thumb, inverting, and easing off the thumb slightly. The appropriate iodine solution (three, five, or fourteen day, according to the dip being tested) is then poured in from the stock bottle in amount guessed as well below the total amount required—usually two-thirds of the way up to the word "correct," i.e. the second circular mark. The thumb is then placed over the end of the tube and the contents mixed by inverting two or three times. Addition of iodine is then continued, a few drops at a time, with inversion after each addition, until a faint yellow

colour due to excess of iodine remains permanent for five to ten seconds after mixing. After each mixing the thumb should be slid off the top of the tube sideways, so as to return any adhering liquid to the tube itself. If any doubt exists as to the end-point, a strip of starch paper may be placed over the end of the tube, below the thumb, and allowed to come into contact with the liquid by inclining the tube. A blue coloration imparted to the paper at once indicates excess of iodine. The liquid must of course be mixed before applying the starch test, otherwise a blue coloration will be given by the drop of iodine adhering to the top of the tube from the previous addition.

As a rule, however, the use of starch paper is unnecessary except with very dirty dips. With a very little practice the appearance of the yellow tint due to excess iodine is easily detected, and serves to indicate the end-point with sufficient accuracy.

The level of the fluid is now noted and the quantity of arsenite of soda in ounces, or of water in gallons, to be added per 100 gallons of tank fluid to restore to standard strength, is read directly off the tube. Points lying between graduation marks may be estimated at sight, e.g. on tube C, fig. 1, a point lying between 6 and 9 would be taken as 7 or 8. The test, however, is not sufficiently accurate to render intermediate sight readings of much value. After a few trials it will be found that a sufficient degree of accuracy may be obtained by pouring straight from the stock bottle into the tube. If greater accuracy is required, or if the manipulator has an unsteady hand, use may be made of a small dropping bottle (capacity 20 c.c. and retail cost threepence) included in the outfit. A little iodine from stock may be poured into this, and then allowed into the test-tube drop by drop towards the final end-point. Such a drop-bottle, if used, must be reserved for iodine solution and not dirtied by being used for any other purpose. Care must of course be taken to use the right tube with the right solution.

The amount of fluid in the tank is determined as usual with a measuring stick, and for every hundred gallons present, arsenite of soda or water is added in accordance with the test-tube figures.

As already indicated, the information required for correction of the three standard dips is obtained without calculation or reference to tables, directly from tubes A, B, and C, respectively. When the user of the instrument has adopted an arbitrary standard of his own, tube D must be used along with the iodine solution nearest to the strength he is using. As a rule this will be five-day solution, which gives readings from zero up to 4 lb. of arsenite per 100 gallons. A little calculation is then necessary in order to determine the data for correcting the tank, but this is very simple since the scale of tube D is made to read directly in ozs. of arsenite of soda actually present in 100 gallons of dip. With three-day iodine, each division shows the presence of 1 oz. of *arsenite* per 100 gallons, or .005 per cent. of *arsenious oxide*; with five-day iodine, 2 ozs. or .01 per cent.; with fourteen-day iodine, 3 ozs. or .015 per cent.

Example.—Suppose an arbitrary dip containing $2\frac{1}{2}$ lb. (40 ozs.) per 100 gallons of water is in use at a dipping interval of nine days. Suppose this be tested with tube D and five-day iodine, and the final end-point level stands at the number 17. The amount of arsenite of soda actually present is therefore $17 \times 2 = 34$ ozs. per 100 gallons (or $.01 \times 17 = .17$ per cent. *arsenious oxide*). Since the correct amount should be 40 ozs., it is obvious that 6 ozs. (40–34) must be added to

every 100 gallons of tank fluid to restore to required strength. If on the other hand, the final level in the test-tube is found to stand at the number 24, there are $24 \times 2 = 48$ ozs. of arsenite present. Since the strength wanted is 40 ozs. per 100 gallons, or 1 oz. per $2\frac{1}{2}$ gallons, 8 ozs. too much arsenite is present, and $8 \times 2\frac{1}{2} = 20$ gallons of water, must therefore be added to every 100 gallons of tank fluid to restore to required strength; or, stated otherwise, every 100 gallons must be made up to $100 \times \frac{48}{40} = 120$ gallons.

In the same way any proprietary dip whose concentration is measurable by iodine (e.g. Cooper's) can be corrected to any desired specific strength.

Tube D, showing the actual arsenical content, can obviously also be used for the three standard dips, and in this case a table (Table I) may be used to avoid unnecessary calculation. Tubes A, B, and C are really "luxuries," and are only justified by their convenience and by the lowered risk of slips in calculation or use of the table.

Table I.

In testing 3-day dip, 3-day Iodine must be used.

" 5 " " 5 " " " "

" 14 " " 14 " " " "

Tube Reading.	Composition of tank fluid as determined with.						To correct to standard strength add to every 100 gallons of tank fluid.		
	3-Day Iodine.		5-Day Iodine.		14-Day Iodine.		3-Day Dip.	5-Day Dip.	14-Day Dip.
	Per cent. As ₂ O ₃ .	Ounces of Arsenite in 100 gallons.	Per cent. As ₂ O ₃ .	Ounces of Arsenite in 100 gallons.	Per cent. As ₂ O ₃ .	Ounces of Arsenite in 100 gallons.			
No.							Ounces of Arsenite of Soda		
1	.005	1	.010	2	.015	3	15	30	45
2	.010	2	.020	4	.030	6	14	28	42
3	.015	3	.030	6	.045	9	13	26	39
4	.020	4	.040	8	.060	12	12	24	36
5	.025	5	.050	10	.075	15	11	22	33
6	.030	6	.060	12	.090	18	10	20	30
7	.035	7	.070	14	.105	21	9	18	27
8	.040	8	.080	16	.120	24	8	16	24
9	.045	9	.090	18	.135	27	7	14	21
10	.050	10	.100	20	.150	30	6	12	18
11	.055	11	.110	22	.165	33	5	10	15
12	.060	12	.120	24	.180	36	4	8	12
13	.065	13	.130	26	.195	39	3	6	9
14	.070	14	.140	28	.210	42	2	4	6
15	.075	15	.150	30	.225	45	1	2	3
16	.08	16	.16	32	.24	48	correct	correct	correct
							Gallons of Water.		
17	.085	17	.170	34	.255	51	6 $\frac{1}{4}$	6 $\frac{1}{4}$	6 $\frac{1}{4}$
18	.090	18	.180	36	.270	54	12 $\frac{1}{2}$	12 $\frac{1}{2}$	12 $\frac{1}{2}$
19	.095	19	.190	38	.285	57	18 $\frac{3}{4}$	18 $\frac{3}{4}$	18 $\frac{3}{4}$
20	.100	20	.200	40	.300	60	25	25	25
21	.105	21	.210	42	.315	63	31 $\frac{1}{4}$	31 $\frac{1}{4}$	31 $\frac{1}{4}$
22	.110	22	.220	44	.330	66	37 $\frac{1}{2}$	37 $\frac{1}{2}$	37 $\frac{1}{2}$
23	.115	23	.230	46	.345	69	43 $\frac{3}{4}$	43 $\frac{3}{4}$	43 $\frac{3}{4}$
24	.120	24	.240	48	.360	72	50	50	50
25	.125	25	.250	50	.375	75	56 $\frac{1}{4}$	56 $\frac{1}{4}$	56 $\frac{1}{4}$
26	.130	26	.260	52	.390	78	62 $\frac{1}{2}$	62 $\frac{1}{2}$	62 $\frac{1}{2}$
27	.135	27	.270	54	.405	81	68 $\frac{3}{4}$	68 $\frac{3}{4}$	68 $\frac{3}{4}$
28	.140	28	.280	56	.420	84	75	75	75
29	.145	29	.290	58	.435	87	81 $\frac{1}{4}$	81 $\frac{1}{4}$	81 $\frac{1}{4}$
30	.150	30	.300	60	.450	90	87 $\frac{1}{2}$	87 $\frac{1}{2}$	87 $\frac{1}{2}$
31	.155	31	.310	62	.465	93	93 $\frac{3}{4}$	93 $\frac{3}{4}$	93 $\frac{3}{4}$
32	.160	32	.320	64	.480	96	100	100	100

Example of the use of the Tube D with the Table in Testing Standard Dips.—Suppose a three-day dip is being tested, and after adding iodine (three-day) to starch end-point, the number 12 is registered on the tube. The second vertical column shows the arsenic content as .06 per cent. The third column expresses this as 12 oz. of arsenite of soda per 100 gallons of dip. The eighth column shows that 4 ozs. of arsenite of soda must be added to every 100 gallons of tank fluid to restore to standard three-day strength.

If, on the other hand, the tube reading were 20 instead of 12, the dip would be indicated as too strong (20 ozs. per 100 gallons), and the eighth column would show that 25 gallons of water would have to be added to every 100 gallons of tank fluid to restore to standard strength.

If the number 16 were registered on the tube the dip would be indicated as correct, or of standard strength, 16 ozs. of arsenite per 100 gallons or .08 per cent. As_2O_3 .

It is obvious that a similar table (of two columns) can easily be constructed for any arbitrary dip at all. Inspection of Table I at once shows how this can be done. Suppose the arbitrary dip in use contained 20 ozs. of arsenite per 100 gallons and it was desired to maintain it at this strength. The fluid could be tested either by three-day or five-day iodine—three-day being nearest. With the latter iodine solution the table would then run :—

Tube reading using 3-day Iodine.	To correct to required strength, add to every 100 gallons.
	Ounces of Arsenite of Soda.
1	19
2	18
3	17
—	—
—	—
—	—
—	—
19	1
20	Correct
	Gallons of Water.
21	5
22	10
23	15
—	—
—	—
—	—
—	—
31	55
32	60

After having corrected any dip to required strength by addition of the requisite amount of arsenite (dissolved in a little hot water) or by addition of the requisite amount of water, it is advisable to test a fresh sample after *thorough mixing in the tank*, in order to make sure that correction has been properly effected.

With a little care, and with the use of the drop-bottle if any difficulty is experienced in pouring direct from the stock iodine bottle, the end-point can be obtained to half a division, i.e. to within $1/32$ nd, or 3 per cent. This degree of accuracy is more than sufficient for practical purposes. Indeed, if on testing a tank the dip is found to be within one division above or below standard strength, correction is scarcely worth while.

The test is in any case only approximate. As a rule the arsenite found by the dip-tester is a little higher than that found by accurate laboratory analysis, and the dip therefore appears to be a little stronger than it really is. The tank is always contaminated by urine and faeces from the cattle passing through, and such foreign matter itself registers to iodine as if it were arsenite of soda. This error cannot be avoided in a rough field test, and is not sufficiently constant to be allowed for in making up the standard iodine solutions. It is usually small, however, and even with an old dip rarely exceeds 10 per cent., especially if the end-point on pouring iodine solution into the test-tube is taken as soon as the faint yellow of excess iodine, or blue of starch iodide, remains permanent for a few seconds after mixing. On standing for a minute or more, the first approximately true end-point disappears owing to reaction between iodine and organic matter in the dip. It often happens that an old dip is particularly foul and contains a large amount of solid organic matter. Although soluble organic matter reacts more readily with iodine than does insoluble, it is still preferable to filter the dip before testing. The end-point is more easily detected and the results somewhat more accurate. A packet of filter papers (one folded to indicate the method of using) and a funnel, may therefore advantageously be included in the outfit. Filtration, if carried out, may be made from the mixing bottle direct into the testing tube or from the sampling vessel into the mixing bottle. To obtain a clear filtrate (still coloured, of course), it is necessary to filter the dip twice through the same paper. In nine cases out of ten, however, filtration is superfluous. The filter papers, however, come in useful for removing the insoluble curd (magnesium soap) which is formed by interaction with the magnesium sulphate of the bicarbonate powders in those cases in which soap is a constituent of the dip.

After use, test-tubes should be washed out with clean water and drained before being laid aside. A test-tube brush may therefore also be included in the outfit.

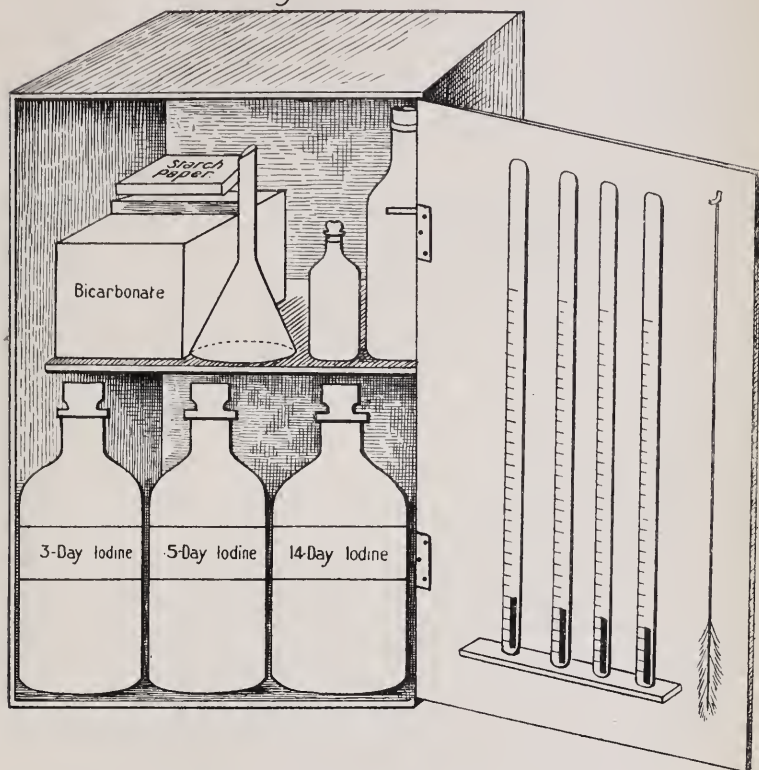
For the sake of keeping the various items of apparatus conveniently together, a cheap cabinet should also be provided. This need only consist of $\frac{3}{4}$ in. wood roughly nailed together, with an interior shelf and a hinged lid.

The whole outfit, as suggested in fig. 2, may be summarized thus:—

- (1) Four graduated tubes.
- (2) Box of bicarbonate powders (2 doz.).
- (3) Book or sheaf of starch papers (about 50)
- (4) Three bottles of iodine solution: 3, 5, and 14 day.
- (5) Corked kali bottle, 4-oz. capacity.

- (6) 20 c.c. dropping-bottle.
- (7) Funnel—about 3-in. diameter.
- (8) Packet of 100 filter papers.
- (9) Test-tube brush.
- (10) Leaflet of instructions for use.
- (11) Rough cabinet.

Figure 2



We may estimate the average production cost of the outfit as follows:—

Iodine Solutions.—45 Litres (a 15-litre bottle of each) may be made up and standardized in three hours at a cost of 17s. for material and, let us say, £1 for the work. This gives a production cost of 10d. a litre or less than 3d. a bottle (8 oz. or about 230 c.c.). The bottles cost 4d each retail. The production price is therefore only 7d. per bottle.

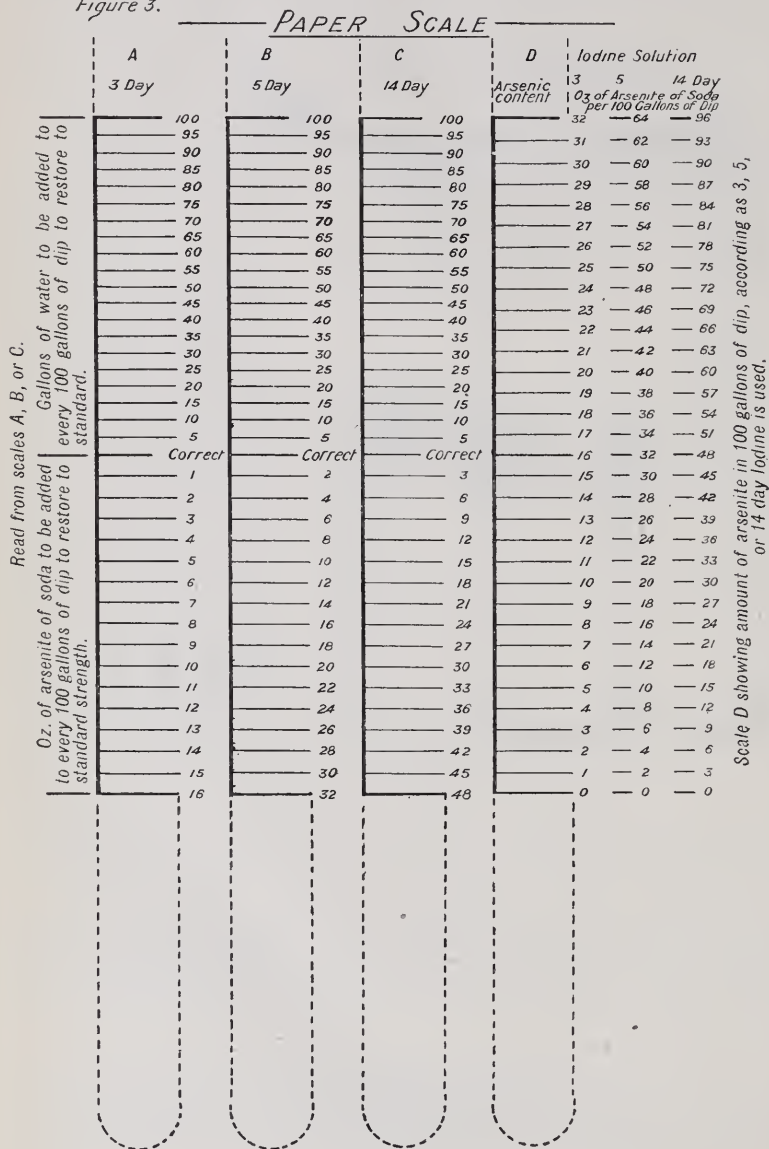
Bicarbonate Powders.—Sodium bicarbonate and magnesium sulphate, pure, are each sold retail at 6d. a lb.—sufficient for 150 to 200 tests. Made up into the form of powders in cardboard boxes of two dozen, the cost of material would be below 1d. per box. Allowing

for folding and boxing, the cost of production would be below 6d., and the powders could therefore easily be sold retail at 1s. per box.

Starch Papers.—These can already be bought retail at 2d. per book or 1s. 6d. per box of a dozen books.

Items 5, 6, 7, and 8 are sold retail at a few pence each.

Figure 3.



Testing Tubes.—The cost of these is difficult to estimate. As previously mentioned, very similar tubes graduated from top to bottom in intervals of $\frac{1}{2}$ c.c. are already sold retail at 8½d. each. The cost of graduating the test-tubes required for dip-testing would not be high, but the fact that they are inscribed with wording, and would be made in limited numbers, would probably bring the cost up to about £8-£10 per 100.

Cabinet.—Since this merely serves as a convenient cupboard to keep the apparatus together, its get-up could be as cheap as possible. An order for about 100 would probably be executed for about £10.

Totalling the various items together brings the cost of production of the whole outfit (fig. 2) to about 15s. It could therefore be sold retail at from 25s.-30s.

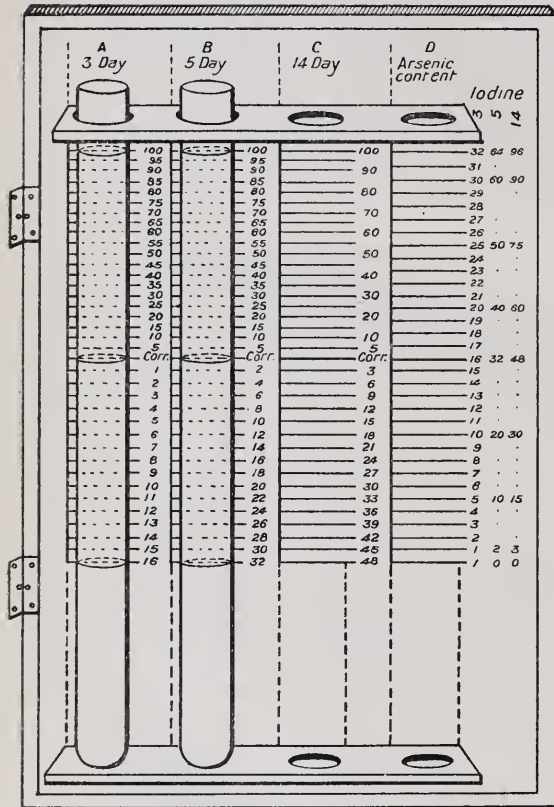
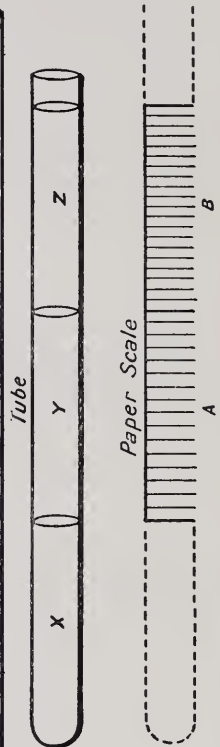
For replenishing purposes, spare tubes could be sold at 3s. or 4s. each; iodine solutions is. 6d. a bottle or about 2s. 6d. or 3s. a litre (four bottlefuls) if bought in bulk; starch papers at 3d. a book; and reaction powders at 1s. a box of 24. A reasonable retail price for consumable materials is of even more importance than the prime cost of the instrument.

Alternative in Scale Construction.—In this outfit the four graduated test-tubes are assessed as representing half the whole cost of production. If this is actually found to be the case, it might be found cheaper to print the graduation system upon a sheet of stout paper protected by a pyroxylin varnish and to paste this on to the lid of the box in such a way that when plain ungraduated tubes were placed in front, the level of the liquid could be directly read off behind. Fig. 3 indicates the form of paper scale which would then be required. Some sort of a supporting rack, either of sheet-iron or of wood, on the principle of an ordinary test-tube stand, would then be required in place of the wooden pegs of fig. 2. Fig. 4 shows the lid of the cabinet arranged to hold the tubes. Since the test-tubes A, B, C, D of fig. 1 would now be all the same and therefore interchangeable, only one (instead of four) would be actually needed, although a second might be included. The tube itself, although ungraduated in detail, would still have to be scratched at three points corresponding to the three major divisions of the paper scales in such a way as to be divided into three equal capacities. This is necessary in order that the test-tube may be accurately "set" in front of the scale. In fig. 4b an isolated tube is shown. X, Y, and Z would have to be of equal capacity, and the lengths Y and Z always the same (say, 8 cm.) and equal to *a* or *b* of the paper scale. Provided the tubes can still slip into the rack, minor variations in bore of different tubes would not matter, although each individual tube would have to be of uniform bore from top to bottom so as to keep the distances between the marks the same. Simple tubes of this description should be very cheap. Unmarked they are sold at 12s. a gross, and marked should not cost more than 6d. each.

In using this simplified appliance the test would be carried out just as already described—alkalinized dip filled to the first mark and iodine solution added with intermittent mixing to starch end-point. The tube would then be placed in the rack in front of the appropriate scale (3, 5, 14 day, or "arsenical content"), care being taken to see that the three markings on the tube coincide with the three major

divisions on the paper scale. The level of the liquid would then be read off and the required information obtained directly. Placed in the first hole of the rack, the correction in ounces of arsenite of soda or gallons of water per 100 gallons of tank fluid, would be indicated for three-day dip. Placed in the fourth hole, the number of ounces of arsenite actually present in the dip would be read off. Similarly the second and third holes would show the correction required for 5-day and 14-day dip, respectively.

Figure 4

Figure 4^b

If this variation in the scheme of graduation effected a marked reduction in the cost of the instrument, it would be worth instituting.

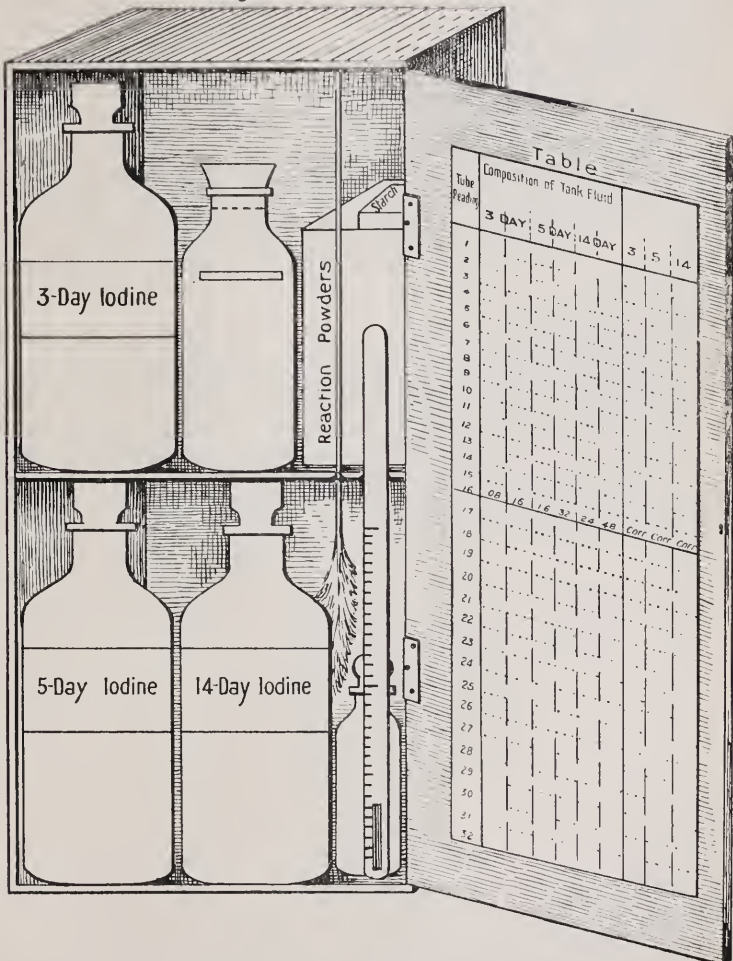
Simplified Tester.—As a third option in the construction of a dip-tester, one graduated tube (D, fig. 1) could be used, along with a reference table (Table I already mentioned) attached to the pamphlet of instructions for use, or pasted on to the lid of the containing box. This outfit (fig. 5) could be simplified to consist of:—

Estimated
production cost.

- (1) Single graduated tube, marked off and numbered in 32 divisions (D) ... 2s. 0d.
 - (2) 4-oz. Kali bottle (1d.), corked and marked ... 0s. 4d.
 - (3) 20-c.c. dropping-bottle ... 0s. 3d.
 - (4) Test-tube brush ... 0s. 2d.
 - (5) Three bottles of iodine solution ... 1s. 9d.
 - (6) Box of bicarbonate powders... 0s. 6d.
 - (7) Book of starch papers ... 0s. 2d.
 - (8) Pamphlet of instructions ... 0s. 6d.
 - (9) Rough containing cabinet... 2s. 0d.
- Total, below 8s.

If sold retail at 15s. complete, it should surely be worth marketing.

Figure 5.



The cost of an *Isometer* without reagents is 35s. *Isometer* testing-fluid is sold at 5s. a bottle; *Isometer* powders in packets of ten at 2s.; *Isometer* testing papers at 1s. a book of about 20. The instrument complete with reagents therefore costs 53s. It appears feasible to suppose that a competitor would find a ready market.

Our own personal concern, however, ends in having carried out official departmental instructions to describe a non-patentable dip-tester marketable at a low cost, and more particularly with consumable reagents involving a low running cost in routine testing.

APPENDIX.

At the time the proofs of this article were being returned to press, the description of the U.S.A. Bureau instrument became available—"Laboratory and Field Assay of Arsenical Dipping Fluids," R. M. Chapin. Bulletin No. 76, U.S. Department of Agriculture.

This instrument is also very simple and compact. A wide-mouth bottle serves as titrating-flask, and a 25 c.c. measuring cylinder, graduated to read both from top to bottom and from bottom to top, takes the place of the burette in ordinary laboratory analysis. 25 c.c. of the dip to be tested is measured from the cylinder into the bottle and one "indicator tablet," containing sodium bicarbonate and soluble starch, is added. The measuring cylinder is then rinsed out with clean water and filled with standard iodine solution. The bottle containing the dip is kept swirling while the iodine is added until the starch-iodide end-point is reached. The quantity of iodine used is then read off. 1 c.c. iodine solution equals one-hundredth per cent. arsenious oxide in the bath.

Any firm proposing to put a cheap instrument on the market would be well advised to read the detailed description of this instrument, which has apparently given every satisfaction in field use. If the American form were decided upon as cheaper than any of the forms described above, tank-correction tables should be supplied for use in South Africa, where the dipping standards are different. Needless to say, the pattern of the instrument is quite immaterial provided that it is cheap, serves the purpose in view, and is adapted for reagents marketable at low cost.

Infectious or Pernicious Anaemia of Equines in South Africa.

By Sir A. THEILER, K.C.M.G., Director of Veterinary Research,
and D. KEHOE, M.R.C.V.S. From the Veterinary Research
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THE object of this article is to call attention to the existence of a disease occurring in horses in South Africa, which disease was not recognized as being definitely existent in this country up to the time when the experiments recorded in this paper were carried out, although it is probable that it has existed here for many years past, hitherto escaping detection through being confounded with other equine affections. It may well have been mistaken, on account of certain of its clinical symptoms, for "biliary fever" (Nuttalliosis, or "Equine piroplasmosis") or for horse-sickness, and more especially that form of this latter disease which is met with in animals which have passed through a previous attack and thereby gained a certain amount of relative immunity, and it may also have been confounded with that condition described by one of us (Sir A. T.) in another place as "ephemeral fever" of the horse, and with other equine diseases occurring in the South African sub-continent.

In this paper it is intended to give in the first place an account of the manner whereby we came to recognize the existence of this disease, then to state the evidence which supports the view that we are dealing with a condition not hitherto definitely recognized in South Africa; next to give the symptomatology of the affection and the post-mortem findings, comparing these with the findings described by writers in other countries; and finally to point out the economic importance of this disease, and this more particularly in connection with the problem it introduces into the preparation from equines of anti-sera intended for combating other equine maladies, in our case the preparation of horse-sickness anti-serum.

The manner in which we first came to recognize the condition will now be entered into, and is as follows:—

In October, 1913, one of us (Sir A. T.) thought it advisable that various samples of horse-sickness virus, which had been collected in this laboratory, should be tested by inoculation into horses which had already passed through an attack, or attacks, of horse-sickness experimentally induced, so as to determine the relative virulency of these newly collected strains of virus. It was hoped that in this way we might gain a knowledge of the properties and peculiarities of these new strains of virus, and that we might find one or other of them more suitable in the immunization of horses against horse-sickness than those which we were then using for this purpose. We also wished to ascertain if there was any one of these strains, and if so which one, which was relatively more virulent than the others and capable of

breaking down the immunity produced by any other of the strains used. At the same time as this plan was projected we had in our possession a number of horses which had been used in the production of horse-sickness anti-serum and were of no further service in this connection, and four other horses immune to horse-sickness. It was, therefore, arranged that we should first take a number (ten) of the samples of virus to be tested, and with each of them inject a number of these immune or hyperimmune horses. It is, perhaps, well to here point out that in this paper and in speaking of horses being "immune" or "hyperimmune" to horse-sickness, we refer by the first term to horses which have passed through one or more attacks of horse-sickness experimentally produced by one or more strains of the horse-sickness virus, and by the second term to horses which, having passed through the disease in the same manner as the first-mentioned animals, have then been subjected to the injection of large quantities of virulent horse-sickness blood with the object of increasing the immunity already possessed by them, and also the production in their body-cells, and subsequent liberation into their blood, of anti-bodies whose action is directed towards the destruction of the horse-sickness virus. The manner in which the hyperimmunization is performed consists in transfusing intrajugularly into a horse already immunized against the disease a quantity of blood from another horse ("virus horse"), which, on account of having been previously inoculated with horse-sickness virus, is passing through an acute attack of the disease, its blood being virulent in a very high degree. The quantity of blood transfused at each operation of "hyperimmunizing" is calculated as being ten litres, and this hyperimmunization may be performed in the case of a given horse on a number of different occasions and at varying intervals. We also speak of a horse being hyperimmune in relation to a certain strain, or strains, of virus. Thus we speak in this paper of horses being hyperimmune to the strains of virus known in this laboratory as Ordinary and Tzaneen, meaning thereby that these animals had been hyperimmunized with the blood of "virus horses" which had derived their infection from one or the other of these two strains of virus. Hereafter we shall refer to horses immune to either the Ordinary or Tzaneen strain as being O. or T. immune respectively, and to horses hyperimmunized against either of these strains as being O. or T. hyperimmune, the numeral employed after these letters being used to denote the number of occasions on which the operation of hyperimmunizing has been performed.

It having been determined to carry out the plan referred to, the first experiment was arranged as follows:—Seventy-six horses, of which fifty-one were O. hyperimmune, twenty-one were T. hyperimmune, three were O. immune, and one was T. immune, were taken and divided up into ten groups, each of which contained horses hyperimmune to the O. and T. strains, and these horses were then inoculated with the ten samples of virus selected for testing, one sample of virus being used for each group of animals. It should be mentioned that the viruses to be tested were either obtained from susceptible horses inoculated at the laboratory with samples of blood collected from cases of horse-sickness occurring under natural conditions in the field in different localities, or (as in the cases of Snyman 72, Steenekamp 73, and Onderstepoort 6025, and others) from horses which had been suffering from horse-sickness, and whose blood had proved to be virulent when injected into susceptible animals at the laboratory.

EXPERIMENT No. 1, 10TH OCTOBER, 1913.

The details of this first experiment made on the 10th October, 1913, are as follows:—

A. Seven hyperimmune horses, 6333 O.5, 6469 O.5, 6470 O.5, 6576 O.5, 6582 O.5, 6314 T.5, and 6418 T.5, were inoculated intrajugularly with 10 c.c. of virus Ziervogel 7483, first generation. (Horse 7483 had contracted horse-sickness and died following inoculation with the blood of a horse which had contracted horse-sickness in Portuguese East Africa in 1912 after having previously passed through an experimentally produced attack of the same disease.)

Result.

None of these horses showed a reaction to this inoculation.

B. Seven hyperimmune horses, 6764 O.5, 6774 O.5, 6785 O.5, 6435 O.4, 6675 O.5, 6425 T.5, and 6432 T.5, were inoculated intrajugularly with 10 c.c. of virus Webster (48) 7764, first generation. (Horse 7764 had contracted horse-sickness and died as a result of infection with the blood of a horse which had contracted the disease in Barberton District, Transvaal, in 1913, although inoculated against this disease in 1911.)

Result.

Of these seven horses, one (6774) showed a reaction from the 5th day and died of horse-sickness on the 10th day; one (6675) showed a reaction from the 4th to the 11th days, the maximum temperature being 104° on the 9th day, and "dikkop" was present on the 8th day; one (6432) showed a temperature reaction from the 7th to the 11th days with a maximum record of 103° on the 9th day; and the other four horses showed no apparent reaction.

C. Seven hyperimmune horses, 6679 O.4, 6846 O.4, 6911 O.4, 7066 O.4, 7077 O.4, 6492 T.5, and 6583 T.5, were inoculated intrajugularly with 10 c.c. of virus McCall (61) 7926, first generation. (Horse 7926 had contracted horse-sickness and died as a result of injection with the blood of a horse which, although inoculated against the disease in 1912, had contracted horse-sickness in the Waterberg District, Transvaal, in 1913.)

Result.

Of these seven horse, one (6583) showed a reaction from the 7th day and died of horse-sickness on the 11th day; one (6679) showed a reaction from the 6th to the 9th days with a maximum temperature of 103° on the 7th and 8th days, with a slight "dikkop" on the left side on the 9th day; and the other five horses showed no apparent reaction.

D. Seven hyperimmune horses, 7325 O.4, 7339 O.4, 7336 O.4, 7346 O.4, 7348 O.4, 6479 T.4, and 6594 T.4, were inoculated intrajugularly with 10 c.c. of virus Rickertsdam (68) 7966, first generation. (Horse 7966 had contracted horse-sickness and died as a result of injection with the blood of a previously immunized horse which contracted the disease in 1913 in the Marico District of the Transvaal.)

Result.

Of these seven horses, one (7325) showed a reaction from the 7th to the 10th days with maximum temperature 104° on the 8th day; one (7336) showed a reaction from the 6th to the 13th days, with

maximum record of 105° on the 11th day and 104° on 8th and 10th days, and 104.4° on the 12th day; one (6479) showed a reaction from the 8th to the 12th days, with maximum 103° on 10th day, and 102.8° on the 11th day; one (6594) showed a reaction from the 6th to the 15th days, with temperature records of 103° and 103.6° on 9th and 12th days respectively, and 102° on the 6th, 8th, and 13th days. The other three horses showed no reaction.

E. Seven hyperimmune horses, 7412 O.4, 7488 O.4, 7592 O.4, 7625 O.4, 7664 O.4, 6599 T.4, and 6601 T.4, were inoculated intrajugularly with 10 c.c. of virus Snyman 72. (The horse from which this virus was obtained had been experimentally immunized against horse-sickness, but contracted an attack of the disease in 1913 in Pretoria District, Transvaal.)

Result.

Of these seven horses none showed a reaction to this inoculation.

F. Seven hyperimmune, and one immune, horses, 7665 O.4, 7668 O.4, 7669 O.4, 7678 O.4, 7145 O.3, 6541 T.3, 6663 T.3, and 8159 O., were inoculated intrajugularly with 10 c.c. of virus McCall (59) 7904, first generation. (Horse 7904 had contracted horse-sickness and died as a result of inoculation with the blood of a horse which, though immunized against the disease in 1912, contracted the disease in 1913 in the Waterberg District, Transvaal.)

Result.

Of these eight horses, one (7665) showed a reaction commencing on the 15th day; one (7678) showed a reaction commencing on the 14th day; one (6663) showed a reaction to 103° on the 15th day, and a further reaction from the 19th day, but as the subsequent inoculation of all horses was made on the 17th day, these reactions will be dealt with later in connection with the second experiment. Another horse (6541) showed a reaction to 104° and 105° on the 13th and 14th days respectively, and a further reaction which commenced on the 17th day, and which will be dealt with in the same manner as in the last three cases. Finally, one horse (7669) showed a reaction from the 5th to the 10th days, with maximum temperature of 104° on the 6th and 7th days. The remaining three horses showed no apparent reaction to the inoculation.

G. Seven hyperimmune, and one immune, horses, 7293 O.3, 7156 O.3, 7257 O.3, 6766 O.5, 7318 O.3, 6680 T.3, 6698 T.3, and 8131 O., were inoculated intrajugularly with 10 c.c. of virus Steenekamp 73. (The horse from which this virus was obtained had been experimentally immunized against horse-sickness, but contracted an attack of the disease in 1913 in Pretoria District, Transvaal.)

Result.

Of these eight horses none showed a reaction to this inoculation.

H. Seven hyperimmune, and one immune, horses, 7326 O.3, 7327 O.3, 7076 O.3, 7320 O.2, 7410 O.1, 6707 T.3, 6732 T.3, and 7939 O., were inoculated intrajugularly with 10 c.c. of virus Onderstepoort 6025. (This virus was obtained from a horse previously immunized against horse-sickness, and which contracted the disease in 1913 on the farm Onderstepoort, Pretoria District, Transvaal.)

Result.

Of these eight horses, one (7410) showed a reaction from the 5th day and died of horse-sickness on the 8th day; one (7327) showed a reaction from the 6th to the 15th days, with maximum record of 105.6° on the 12th day, and 104° on the 8th, 11th, and 13th days, and "dikkop" on the 12th day; one (6732) showed a reaction from the 5th to the 10th days with records of 103° and 104° on the 6th and 7th days respectively; one (7076) showed a reaction from the 7th to the 15th days, with maximum records of 103° and 103.6° on the 7th and 11th days respectively; one (7320) showed a reaction from the 5th to the 9th days, the maximum record being 104° on the 7th and 8th days; and, finally, one (7326) showed a reaction from the 5th to the 9th days, with maximum record of 104° on the 6th, 7th, and 8th days. The remaining two horses showed no apparent reaction.

I. Seven hyperimmune, and one immune, horses, 7413 O.3, 7417 O.2, 7442 O.1, 7444 O.1, 7446 O.1, 7228 T.3, 7331 T.3, and 8234 T., were inoculated intrajugularly with 10 c.c. of virus Edgar (88) 8200, first generation. (Horse 8200 had contracted horse-sickness and died, following inoculation of blood of a horse alleged to be naturally "salted" to horse-sickness, and which contracted the disease in 1913 in the Zoutpansberg District, Transvaal.)

Result.

Of these eight horses none showed any apparent reaction to this inoculation.

J. Nine hyperimmune horses, 7455 O.1, 7490 O.2, 7503 O.1, 7677 O.2, 7088 O.2, 5971 O.6, 4177 T.7, 5782 T.6, 6328 T.6, and two susceptible control horses (8259 and 8408), were inoculated intrajugularly with 10 c.c. of virus Bulawayo 7245, twelfth generation. (This virus was originally obtained from a case of horse-sickness occurring in Bulawayo some years ago, and had been passed through twelve horses at the laboratory since that time.)

Result.

Only one of the hyperimmune horses showed a reaction following this inoculation, and this was only indicated by a record of 102° on the 5th and 6th days.

The control horses contracted horse-sickness and died, one (8259) on the 5th, and the other (8408) on the 6th day.

Discussion of Results.

From the results of the foregoing experiment, it appeared that the ten viruses used could be divided into two groups depending upon their possession, or otherwise, of the property of inducing reactions in the animals into which they were inoculated. Thus we can include in one group the five viruses Ziervogel 7483, first generation; Snyman 72, Steenekamp 73, Edgar (88) 8200, first generation, and Bulawayo 7245, twelfth generation, since these did not cause reactions in the inoculated horses; and into the other group we can place the other five viruses, Webster (48) 7764, first generation; McCall (61) 7926, first generation; Rickertsdam (68), first generation; McCall (59) 7904, first generation; and Onderstepoort 6025, since these caused the appearance of reactions in the horses inoculated with them, and in the case of Onderstepoort 6025, McCall (61) 7926, and Webster (48) 7764, it was seen that each of these viruses, in at least one case in each of the groups of horses into

which they were inoculated, was capable of not only causing a temperature reaction to appear, but even to produce death from horse-sickness, and in another horse in each of their groups they produced a reaction accompanied by dikkop.

It should be noted that the horses reacting to the inoculation were bled at varying periods during their reactions, as it was further considered desirable to test the properties of the viruses so obtained.

The next experiment made was carried out on the 27th October, 1913, i.e. 17 days from the time when the first inoculation was made, and the general plan laid down was as follows: It was decided, in the first place, that certain of the viruses obtained from reacting horses in the manner stated in the last paragraph should be tested, and that these tests should be performed on horses of the reacting groups other than the group from which the virus to be tested was obtained, and when one of these viruses had been obtained from a horse which had only shown a reaction, it was also injected into a control animal in order to prove its capability or otherwise of producing horse-sickness. In the second place, it was decided that the groups of horses which had shown no reactions to the viruses with which they were inoculated should be now tested with samples of strains of virus which had not been previously tested on immune or hyperimmune animals. Thus the second experiment was laid down in detail as follows:—

Eight of the viruses obtained from horses reacting to the previous inoculation were taken, and these were—

(1) Rickertsdam (68), second generation. Horse 7336 bled on 18/10/13.

(2) Rickertsdam (68), second generation. Horse 6479 bled on 21/10/13.

(3) Webster (48), second generation. Horse 6774 bled on 17/10/13.

(4) McCall (61), second generation. Horse 6679 bled on 18/10/13.

(5) McCall (61), second generation. Horse 6583 bled on 19/10/13.

(6) Onderstepoort (6025), first generation. Horse 7410 bled on 17/10/13.

(7) Onderstepoort (6025), first generation. Horse 6732 bled on 18/10/13.

(8) McCall (59), second generation. Horse 7669 bled on 17/10/13.

Viruses Nos. (3), (5), and (6) of this group had been obtained during the reactions of horses which died from horse-sickness as a result of the inoculation, and hence there was no need to inject them into control animals in order to prove their virulency. The other five viruses, however, being obtained during the reactions of horses which did not die from horse-sickness as a result of the inoculation, were injected into control animals at the same time as they were inoculated into the hyperimmune horses. These eight viruses were injected into the following hyperimmune horses whose numbers are here given, and the letters used after these numbers refer to the groups in which these horses were placed during the first inoculation.

EXPERIMENT NO. 2, 27TH OCTOBER, 1913.

K. Virus Rickertsdam 7336 (18/10/13) was injected intrajugularly, in the quantity of 10 c.c., into six hyperimmune horses.

Nos. 7077C, 6435B, 7076H, 7145F, 6679C, 8159F, and into a control horse, 8372, which happened to be O. immune.

Result.

The control horse, 8372, died of horse-sickness on the 7th day. Of the six hyperimmune horses, four (Nos. 7077, 6435, 6679, and 7076) showed no apparent reaction to this inoculation, whilst the other two reacted and one died, the death of this horse at that time being attributed to "biliary fever." Thus No. 7145 showed a reaction from the 8th to the 10th days with maximum records of 104° on the 9th and 10th days, and No. 8159 showed a reaction from the 14th day up to the time of death on the 22nd day, and with maximum points of 104° , 103.2° , 103.6° , and 104° on the 15th, 17th, 18th, and 20th days.

L. Virus Rickertsdam 6479 (21/10/13) was injected intrajugularly, in the quantity of 10 c.c., into three hyperimmune horses, Nos. 6732H, 6432B, 6492C, and into a control horse, 8302, which happened to be T. immune.

Result.

The control horse, 8302, died of horse-sickness on the 9th day. Of the three hyperimmune horses, one (No. 6492) showed a well marked reaction from the 4th to the 13th days, with maximum records of 104.4° , 104° , 104° , 105.2° , and 103.8° on the 6th, 7th, 9th, 10th, and 11th days respectively; one (No. 6732) only showed an elevation to 104.6° and 102.6° on the 8th evening and 9th morning respectively; the third horse (No. 6432) showed no apparent reaction to the inoculation.

M. Virus Webster 6774 (17/10/13) was injected intrajugularly, in the quantity of 10 c.c., into five hyperimmune horses, Nos. 7336D, 7066C, 7320H, 7665F, 7939H.

Result.

Of these horses, one (No. 7066) showed no reaction, and three showed no definite reactions, but slight transient temperature elevations as follows: No. 7336 showed a record of 102.2° and 102° on the 7th and 12th evenings respectively; No. 7320 showed a record of 103° and 102° on the 3rd and 12th evenings respectively; No. 7939 recorded 102° and 102.2° on the 6th and 12th evenings respectively; and in the case of the fifth horse (No. 7665) the elevation in the temperature noted following this second inoculation could now be seen to be only a continuation of the reaction which had commenced two days previous to this inoculation and fifteen days after the first injection of virus, and it was apparent that this reaction was produced by the virus used for the first inoculation [7904, McCall (59), first generation]. This reaction, commencing two days before the second injection and lasting up to the seventh day following, thus covered a period of ten days, the evening records of the 1st, 2nd, 3rd, 4th, 6th, 8th, and 9th days of the reaction being respectively 103° , 103.8° , 104° , 103° , 103.4° , 104.2° , 103.4° .

N. Virus McCall 6679 (18/10/13) was injected intrajugularly, in the quantity of 10 c.c., into the five hyperimmune horses, Nos. 7325D, 7326H, 6764B, 7668F, 7678F, and into a control horse, No. 8373, which happened to be O. immune.

Result.

The control horse, 8373, died of horse-sickness on the 8th day. Of the hyperimmune horses, four (Nos. 7325, 7326, 6764, and 7668) showed no reaction, whilst in the case of the fifth (No. 7678) the condition seen was similar to that in the case of horse 7665, mentioned above in the immediately preceding group of horses, since the reaction present on the day of and lasting up to the 13th day after the second inoculation could now be seen to be a continuation of the reaction which set in on the 14th day after the first inoculation and was thus produced by the virus used at the first inoculation, i.e. virus McCall (59) 7904, first generation. The total number of days covered by this reaction was 17, and some idea of the nature of reaction may be conveyed by stating the evening records of the 1st, 2nd, 3rd, 4th, 6th, 7th, 10th, 12th, and 14th days, since these records of 103.6°, 103.8°, 104.2°, 105°, 103.4°, 103.4°, 104.8°, 103.6°, and 104.2° represent the highest points of the temperature curve.

O. Virus McCall 6583 (19/10/13) was injected intrajugularly, in the quantity of 10 c.c., into the three hyperimmune horses, Nos. 6594D, 6707H, and 6663F.

Result.

Of these three horses, one (No. 6594) showed a reaction from the 14th to the 25th days, with a slight remission on the 17th, 18th and 19th days, the highest points of the curve being 104.8°, 103°, 102°, 103°, 104°, 104.4°, 104.8°, 104°, and 103.2° on the 14th, 16th, 18th, 19th, 20th, 21st, 22nd, 23rd, and 24th days respectively; one (No. 6707) showed a rise to 103.4° on the 3rd day and then a reaction from the 14th to 16th days, the records on the 14th evening, 15th morning and evening, and 16th morning being respectively 103.2°, 103°, 103°, and 103.2°; the third horse (No. 6663) showed a reaction which commenced on the 2nd day and lasted up to the 7th day, the highest points of the curve being 103.6°, 103.4°, 103.8°, 104°, and 103° on the 3rd, 4th, 5th, 6th, and 7th days respectively, but in the light of the experience of Horses Nos. 7665 and 7678, and seeing that in the case of this horse there was an evening record of 103° on the 2nd day preceding this second inoculation, the reaction here mentioned must have been produced by the virus used in the first inoculation, namely, virus McCall (59) 7904, first generation.

P. Virus Onderstepoort 7410 (17/10/13) was injected intrajugularly, in the quantity of 10 c.c., into five hyperimmune horses, Nos. 7339D, 6911C, 6785B, 7669F, and 7346D.

Result.

Two of these horses (Nos. 6785 and 7346) showed no reaction; one (No. 6911) only showed a rise of temperature on one occasion, the 7th day, and to 102°; another (No. 7669) showed an evening record of 104.6° on the day of inoculation and elevations to 104.2°, 102°, and 103° on the 6th, 7th, and 9th evenings respectively [and it should be noted that this animal had, previous to this second inoculation, been reacting to the injection of the first virus McCall (59) 7904, first generation]. The fifth horse (No. 7339) showed no reaction up to the 17th day, when the third inoculation was made, but the reaction commencing on the day following this third injection, and therefore nineteen days after the injection of the above virus (Onderstepoort 7410), would seem to be due to this latter virus, the period of one

day appearing to be too early to allow of the third virus producing its effects. This reaction will be again referred to in connection with the inoculation of this horse with the third virus [Dunning (55) 6680, second generation, 5/11/13].

Q. Virus Onderstepoort 6732 (18/10/13) was injected intrajugularly, in the quantity of 10 c.c., into three hyperimmune horses, Nos. 6479D, 6425B, and 6541F, and into a control horse, No. 8306, which happened to be T immune.

Result.

Of the three hyperimmune horses, one (No. 6479), showed a slight tendency to react between the 5th and 8th days, the evening records of the 6th, 7th, and 8th days being 102° , 103° , and 102° respectively; another (No. 6425) showed an elevation to 102° on the 5th evening, and again a reaction which, commencing on the 15th day and continuing over the time of the subsequent inoculation of this horse, which was made on the 17th day, lasted up to the 13th day after this latter (third) inoculation, and will be again referred to in this connection. [See inoculation on 13th November with virus Martin 6766 (6/11/13).] The third horse (No. 6541) showed a reaction on the day of the inoculation with virus Onderstepoort 6732 (18/10/13), but, since there had existed a reaction on the 4th and 3rd days previous to this inoculation, with evening records of 104° and 105° respectively, it was evident that this reaction, which lasted up to the 10th day following the second inoculation, had to be attributed to the virus used for the first injection, namely, virus McCall (59) 7904, first generation. This reaction, commencing as it did four days previous to and persisting up to the 10th day subsequent to this second inoculation, thus covered a total period of 15 days, and during this time the higher points of the temperature curve were 104° , 105° , 103° , 102.8° , 104.8° , 105.2° , 105° , 105.6° , 105.2° , 105° , 104.2° , 103.6° , and 103.6° on the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th, 10th, 11th, 13th, and 14th days.

The control horse, No. 8306, which was T. immune, did not die of horse-sickness as was hoped for, but only showed a reaction between the 4th and 7th days, with evening records of 102.4° , 103.4° , and 103° , on the 4th, 5th, and 6th evenings, and, again, 102° on the 9th evening; a further slight tendency to react on the 21st and 22nd days was indicated by the respective evening records of 101.6° and 101.8° . It was again inoculated on the 37th day with 10 c.c. intrajugularly of the "sister" virus Onderstepoort 7410 (17/10/13), which proved its susceptibility to horse-sickness by causing its death on the 11th day.

In view of this result it was decided to inject again the virus Onderstepoort 6732 (18/10/13) into a control horse, and for this purpose a non-immune horse, No. 8366, was selected and injected with 10 c.c. of the virus intrajugularly. This horse also did not die within the expected time, but instead only showed a reaction on the 15th, 16th, and 17th days, with evening records of 102° , 105.2° , and 103.8 , and with a temperature of 103° on the 21st day. In order to test its susceptibility to horse-sickness, it was injected on the 28th day intrajugularly with 10 c.c. of the "sister" virus Onderstepoort 7410 (17/10/13) as was done also in the last case, and this injection caused the death of the animal from horse-sickness on the 10th day.

The blood of these control horses taken during the time they were passing through these reactions was tested by inoculation into susceptible horses in the quantity of 10 c.c. injected intrajugularly. The blood of No. 8306, injected into horse No. 8471, produced a reaction in this latter animal, first from the 6th to the 11th days, with evening records of 102.6°, 104.8°, 103.4°, 103.8°, 103.6°, 102°, on the days included in this period, and, again, a reaction which, commencing on the 15th day, terminated with the death of the animal on the 25th day. *Nuttallia equi* was present in the blood on the 22nd day, and the death of this horse was attributed to "biliary fever." The blood of No. 8366, injected into horse No. 8460, did not produce any apparent reaction in this latter animal.

R. Virus 7669 (17/10/13) was injected intrajugularly, in the quantity of 10 c.c., into four hyperimmune horses, Nos. 6675B, 6846C, 7348D, 7327H, and into a control horse, No. 8414, which happened to be O. immune.

Result.

Of the four hyperimmune horses, one (No. 6675) showed no reaction to the inoculation; one (No. 7327) showed a rise to 103.6° on the 3rd evening, but otherwise did not react further; one (No. 6846) showed a rise to 102° on the 12th evening, and no further reaction up to the 17th day, when this horse was inoculated for the third time. Since, however, a reaction occurred from the 2nd to the 6th days following this third injection, it would appear that the above virus 7669 (14/10/13) of the second inoculation was responsible for this reaction, assuming a 19 days' period of incubation in this case rather than to assume it as due to the third virus (and this also for other reasons given later) with a two days' incubation period. The fourth horse (No. 7348) showed a reaction which, commencing on the 17th day, lasted up to the 31st day, when the animal died. The highest points of the curve were noted on the 17th, 18th, 20th, 21st, 24th, 26th, and 30th days, being 105°, 104.4°, 105°, 105.4°, 105.2°, 104.2°, 105.2°, and 104°, respectively. The death of this animal on the 31st day was at that time attributed to broncho-pneumonia.

The control horse, No. 8414, which was O. immune, showed no rise of temperature (except that to 102.6° on the 14th evening) up to the 37th day. [It was then inoculated intrajugularly with 10 c.c. of the "mother" virus McCall (59) 7904, first generation, and this virus proved its susceptibility to horse-sickness by causing its death from this disease on the 10th day following this latter injection.]

It may also be mentioned that virus 7669 (17/10/13), on being injected later into another control horse, No. 8371, which was not immune to horse-sickness, only gave indications of a slight tendency to cause a reaction, as indicated by records of 103.6° on the 11th day, 101.8° and 101.4° on the 19th and 20th days respectively, and 101.6° on the 22nd day. It was inoculated again on the 28th day intrajugularly with 10 c.c. of virus 7348 (13/11/13), which was obtained from horse 7348 whilst passing through the reaction produced by virus McCall (59) 7669 (17/10/13) (see immediately above). The only reaction following this injection was noted on the 5th and 6th days, the evening records being 103.8° and 102.8° respectively, but otherwise no reaction occurred up to the 26th day, when the horse was reinoculated with the same virus as used on the last occasion, 7348 (13/11/13), the dose this time, however, being 100 c.c., also given

intrajugularly. This inoculation, however, did not cause the production of horse-sickness, but following it there were observed a series of reactions, as on the 2nd day to 103.8° , on the 22nd and 24th days to 104° and 104.2° respectively, on the 31st, 33rd, 34th, and 38th days, to 102.6° , 103.2° , 103° , and 102.6° respectively; on the 54th, 56th, 57th, and 58th days to 103.6° , 102.8° , 103° , and 103.2° respectively; and on the 108th, 109th, and 110th days to 106° , 105° , and 103° respectively.

Discussion of Results.

Taking these results into consideration along with those of the first experiment, we must call attention to the behaviour of those seven horses which had been injected in the first experiment with the virus McCall (59) 7904, first generation. Of these there are three which do not call for special comment, namely, Nos. 7145, 7668, and 7669, which in the second experiment will be found in the lots of horses inoculated with the viruses 7336, 6679, and 7410 respectively, but in the case of the other four horses, namely, Nos. 7665, 7678, 6541, and 6663, referred to in the above second experiment, in the lots inoculated with viruses 6774 (17/10/13), 6679 (18/10/13), 6732 (18/10/13), and 6583 (19/10/13), special reference is made to their reactions, and it is pointed out that these reactions must really be considered as being due to the previous injection with virus McCall (59) 7904, first generation. It may here be added that, noting these peculiar reactions whilst the second experiment was in progress, we decided to test the virus McCall (59) 7904, first generation, on a susceptible horse. (This was done on the 6th November, 1913, 3 c.c. being injected intrajugularly into horse No. 8422, and as a result a reaction set in from the 6th day, and death from horse-sickness occurred on the 11th day.) The virus 7669 (17/10/13) used in this second experiment, and which is the "daughter" virus of 7904, had not produced any definite reactions up to the 17th day following the injection, that is to say, at the time when we considered the results of the second experiment, but we also noted the fact that this virus did not kill the control horse (No. 8414) with horse-sickness, and so we decided to test this horse with the "mother" virus 7904, and to inject another control horse, No. 8371. (The results of these later injections have been given above merely for the sake of convenience, and not deferred until later as they should be seeing that we are viewing these experiments and their findings in the chronological sequence of events.) We also noted that a reaction to virus 7669 seemed to be commencing in the horse No. 7348. The reactions produced by the virus McCall (59) 7904, first generation, were rather puzzling because of their long incubation period and the clinical symptoms shown by the horses during the reactions, since the general appearance of the animals, the appearance of their visible mucous membranes, the relations between the degree of the fever and the pulse rate, and the general absence of definite symptoms of either horse-sickness or biliary fever, led us to think that we were not dealing with the varieties of those diseases hitherto familiar to us, and, whilst undecided as to whether or not the reactions were only indicative of a modified form of horse-sickness, due perhaps to the fact that the inoculated animals were hyperimmune to the O. and T. strains of horse-sickness virus, we decided to keep an open mind on the subject and await further happenings.

The behaviour of the other viruses used did not at this stage call for special comment, except in the case of the virus 6732

(18/10/13), which failed to produce horse-sickness in the control horse (No. 8306), and on this account we decided to inject this virus into a fresh control horse (No. 8366), and to test the susceptibility of the horse No. 8306 by injecting it with the "sister" virus 7410. (The results of these injections have, however, for the sake of convenience, been already referred to above.)

At this point it must now be recalled that in connection with the experiment No. 2 still under discussion it was also decided to introduce for purposes of testing several samples of strains of virus not hitherto tested on immune or hyperimmune animals, and it was decided that these strains should be tested on lots of horses drawn from those groups which did not show reactions to the first inoculation, and so, on the same date (27th October, 1913) as the last portion of this experiment was made, and seventeen days from the time of the first injection, the inoculations with these new samples were carried out. Eight lots of horses were drawn from the previously non-reacting groups, and as far as possible these lots included one or more representatives of each of these groups. The viruses used were obtained, and their virulency tested, in the same way as that mentioned in dealing with the strains used in the first experiment. The eight viruses used were the following:—

(1) Virus Martin (52) 7862, first generation. (Horse 7862 had contracted horse-sickness and died as a result of inoculation with the blood of a horse which in 1913 contracted the disease in the Tugela District, Natal.)

(2) Virus York (69) 7950, first generation. (Horse 7950 had contracted horse-sickness and died as a result of inoculation with the blood of a horse which in 1913 contracted the disease in Onderstepoort, Pretoria District, Transvaal.)

(3) Virus Brown 78. (This virus was obtained from a horse contracting and dying from horse-sickness in 1913 in the town of Pretoria, Transvaal.)

(4) Virus Wernick (79) 8096, first generation. (Horse 8096 had contracted horse-sickness and died as a result of inoculation with the blood of a horse which contracted the disease in the vicinity of Onderstepoort, Pretoria District, Transvaal.)

(5) Virus Dunning (55) 7872, first generation. (Horse 7872 had contracted and died from horse-sickness as a result of inoculation with the blood of a mule which, inoculated against the disease in December, 1912, had contracted and died from horse-sickness in February, 1913, in the Lydenburg District, Transvaal.)

(6) Virus Edgar (63) 7937, first generation. (Horse 7937 had contracted and died from horse-sickness as a result of inoculation with the blood of a mule which contracted the disease in 1913 in the Zoutpansberg District, Transvaal.)

(7) Virus Mule Inspector O. W. 75. (This virus was obtained from a mule which contracted horse-sickness in 1913 in the vicinity of Onderstepoort, Pretoria District, Transvaal.)

(8) Virus Onderstepoort 7781. (This virus was obtained from a mule which was inoculated against the disease, but which naturally contracted the disease in 1913 on the farm Onderstepoort, Pretoria District, Transvaal.)

The above viruses were inoculated into the following horses with the following results. The letters used after the numbers of the animals have the same significance as that referred to in connection with the other portion of this experiment.

S. Virus Martin (52) 7862, first generation, was injected intrajugularly, in the quantity of 10 c.c., into the six hyperimmune horses, Nos. 7677J, 7413I, 6766G, 7412E, 6328J, and 8234I.

Result.

Of these six horses one (No. 6766) showed a reaction which, commencing on the 8th day, lasted up to the 13th day, the highest points of the curve being 103.6° , 103.8° , 104° , and 104° , these occurring on the 8th, 9th, 10th, and 11th days respectively, and "dikkop" appearing in the left supraorbital region on the 13th day; another horse (No. 6328) showed a reaction from the 5th to the 11th days, the highest points of the curve being 103° , 103.6° , 104° , 102.8° , and 102.8° on the 5th, 6th, 7th, 8th, and 11th days respectively. The other four horses showed no definite reaction, the only rises in temperature noted being transient. Thus No. 7677 showed evening records of 102° and 103.2° on the 7th and 9th days, No. 8234 showed on the 9th and 12th evenings 104° and 102° respectively, No. 7412 showed 102° on the 7th evening, and No. 7413 102.2° on the 4th evening, otherwise they showed no temperature elevation.

T. Virus York (59) 7950, 1st generation, was injected intrajugularly, in the quantity of 10 c.c., into the six hyperimmune horses, Nos. 6333A, 7503J, 7417I, 7156G, 7228I, and 5782J.

Result.

Of these six horses one (No. 7228) showed a reaction commencing on the 5th day, and death from horse-sickness occurred on the 11th day. Two horses (Nos. 7156 and 7417) showed no rise in temperature, and the other three showed only transient elevations, but no definite reactions. Thus No. 6333 showed 102.8° on the 4th evening, No. 7503 102° on the 11th and 12th evenings, and No. 5782 102° on the 10th and 12th evenings.

U. Virus Brown 78 was injected intrajugularly, in the quantity of 10 c.c., into the six hyperimmune horses, Nos. 7488E, 6469A, 7490J, 7442I, 4177J, and 7331I.

Result.

Of these six horses one (No. 7331) showed a reaction from the 4th to the 10th days, the higher points of the curve being 102.2° , 104.2° , 103.4° , 103° , 104° , and 102.6° on the evenings of the 4th, 5th, 6th, 7th, 8th, and 9th days respectively; one (No. 7490) showed a reaction from the 6th to the 9th days, the higher records being 102.4° , 101.8° , and 103° on the 6th, 7th, and 8th days respectively; another (No. 7488) showed a reaction from the 5th to the 9th days, the higher records being 103.8° , 104.8° , 104.6° , and 104° on the 5th, 6th, 7th, and 8th evenings respectively. Of the remaining three horses, No. 7442 showed a transient rise to 103° on the 6th evening, but otherwise this horse, as well as Nos. 4177 and 6469, showed no reactions.

V. Virus Wernick (79) 8096, first generation, was injected intrajugularly, in the quantity of 10 c.c., into the five hyperimmune horses, Nos. 7257G, 7592E, 6470A, 6698G, and 6599E.

Result.

Of these five horses one (No. 6698) showed a slight reaction; the highest temperatures, however, were only 102.6° , 102.8° , and 103° on the 8th, 12th, and 14th evenings respectively; the other four horses (Nos. 7357, 7592, 6470, and 6599) showed no reactions.

W. Virus Dunning (55) 7872, first generation, was injected intrajugularly, in the quantity of 10 c.c., into the four hyperimmune horses, Nos. 7455J, 7441I, 7293G, and 6680G.

Result.

Of these four horses one (No. 6680) showed a reaction commencing from the 6th day, and death from horse-sickness occurred on the 13th day; one (No. 7455) showed a reaction from the 5th to the 11th days, the higher points of the curve being 104° , 105° , 105° , 104° , and 103.2° on the 5th, 7th, 8th, 9th, and 11th days respectively, and "dikkop" appeared on the 10th day; another (No. 7444) showed a reaction from the 8th to the 14th days, the higher points of the curve being 103.6° , 104° , 105° , 104° , and 103° on the 8th, 9th, 11th, 12th, and 13th days respectively; whilst the fourth horse (No. 7293) showed no rise in temperature save that to 102° on the 7th and 12th evenings.

X. Virus Edgar (63) 7937, first generation, was injected intrajugularly, in the quantity of 10 c.c., into the four hyperimmune horses, Nos. 5971J, 7446I, 7318G, and 6601E.

Result.

Of these four horses one (No. 5971) showed a reaction from the 6th day, and died from horse-sickness on the 11th day; one (No. 7446) showed a rise to 103.6° on the 3rd evening, and then a reaction from the 6th to the 11th days, the higher points of the curve being 102.6° , 102.6° , 103.6° , 103.2° , and 102.6° on the 6th, 7th, 8th, 9th, and 11th evenings respectively; the third horse (No. 7318) only showed a rise to 102° on the 6th and 9th evenings; whilst the fourth (No. 6601) showed no reaction.

Y. Virus Mule Inspector O. W. 75 was injected intrajugularly, in the quantity of 10 c.c., into the four hyperimmune horses, Nos. 7088J, 8131G, 7625E, and 6418A.

Result.

These horses showed no definite reactions to this inoculation, although transient rises to 102° or 103° were shown by each of them on one or two occasions.

Z. Virus Onderstepoort 7781 was injected intrajugularly, in the quantity of 10 c.c., into the four hyperimmune horses, Nos. 6576A, 6582A, 7664E, and 6314A.

Result.

Of these horses none definitely reacted to this injection. No. 7664 showed 103.2° and 103° on the 5th and 8th evenings respectively; No. 6582 showed 105° on the 5th evening; No. 6576, 103° on the 20th evening, and No. 6314 showed no elevation of temperature. As in the last experiment, the horses reacting in the above lots were bled a small quantity (about 200 c.c.) whilst passing through the reactions occurring.

Discussion of Results.

In examining these results on the 13th November, 1913 (in conjunction with those of the other part of this same experiment already discussed), we noted the capability of the three viruses, York (69) 7950, first generation; Dunning (55) 7872, first generation; and Edgar (63) 7937, first generation, to produce death from horse-sickness in one horse in each of the groups into which they were inoculated, and that the Dunning virus produced a reaction accompanied by dikkop in another horse. The other new viruses called for no special comment at this stage, since either they did not produce definite reactions, or they did, and in this latter case it was not certain what the exact nature of these reactions were, and as from the results of the other part of the experiment we had decided to keep an open mind regarding these reactions, we therefore decided to reinoculate all of these horses again. It could also be seen, however, that none of the viruses which had been injected at the first inoculation into the horses used in this part of No. 2 experiment were capable of producing late reactions of a nature similar to that shown by the virus McCall (59) 7904 already referred to.

Now, having all of these results in view, and since we were still attempting to find which virus was relatively the most virulent, we decided to proceed to a third experiment which was planned on the following lines. It was decided to further test the properties of the viruses Onderstepoort 7410 (17/10/13), Webster 6774 (17/10/13), McCall (61) 6583 (19/10/13), obtained from the first inoculation, and the viruses Edgar (63) 5971 (4/11/13), Dunning (55) 6680 (5/11/13), York 7228 (4/11/13), obtained from the second inoculation, since coming as they did from horses which had died of horse-sickness, they were therefore descendants of the most virulent of the viruses hitherto used. We also decided to test the properties of the virus McCall (59) 7348, third generation (13/11/13), since this virus was obtained from a horse inoculated with the virus McCall (59) 7669 (17/10/13), that is to say, a descendant of the virus McCall (59) 7904, first generation, which latter had been noted to produce reactions rather puzzling at this time. This horse, 7348, was, however, the only one of the group inoculated with virus 7669 which was reacting to the injection on the day when the third experiment was made, and the reaction had only been then existent for two days. It can be recalled here that of the eight new strains of viruses introduced in the second experiment, three already referred to produced horse-sickness; two others (virus Onderstepoort 7781 and virus Mule Inspector O. W. 75) produced no appreciable reactions; and the other three [virus Martin (52) 7862, virus Brown 78, and virus Wernick (79) 8096] produced reactions of varying intensity in one or more of the horses into which they were inoculated. Therefore we further decided to test one virus proceeding from six of these lots, and selected for this purpose the viruses obtained from horses injected with these first and last three viruses mentioned, injecting these viruses at the same time into control horses in each case. On account of the reaction in horse 7348 only being in existence for two days on the 13th November, it was thought advisable to postpone the inoculation of horses with this virus for another eight days, until the 21st November, in order to allow this reaction to develop further so that its nature might be observed.

The same hyperimmune horses were utilized for this third experiment, and the selection and arrangement of the lots was made so

that the new viruses, Edgar 5971, Dunning 6680, York 7228, Wernick 6698, Brown 7488, and Martin 6766, were tested on these horses used in the first portion of experiment No. 2 and therefore from groups reacting to the first inoculation, whilst the viruses Onderstepoort 7410, Webster 6774, McCall (61) 6583, and McCall (59) 7348 were tested on horses used in the second portion of experiment No. 2 and thus originally proceeding from those groups which had shown no reactions to the first inoculation.

We also decided at this time to inoculate the control horses, Nos. 8366 and 8371, with the viruses Onderstepoort 6732 (18/10/13) and McCall (59) 7669 (17/10/13) respectively, but this and the results obtained has already been referred to.

The horses, Nos. 6707 and 6594, which, on the 13th November, were reacting to the virus McCall (61) 6583, we decided should not be inoculated at this time, but left over to be utilized later when they had recovered.

We thus proceeded to make the third experiment which, in detail, was as follows:—

The nine viruses used were those hereunder given:—

- (1) Virus Edgar (63), second generation, horse 5971, bled on 4/11/13.
- (2) Virus Dunning (55), second generation, horse 6680, bled on 5/11/13.
- (3) Virus Wernick (79), second generation, horse 6698, bled on 5/11/13.
- (4) Virus Brown (78), first generation, horse 7488, bled on 4/11/13.
- (5) Virus York (69), second generation, horse 7228, bled on 4/11/13.
- (6) Virus Martin (52), second generation, horse 6766, bled on 6/11/13.
- (7) Virus Onderstepoort (6025), first generation, horse 7410, bled on 17/10/13.
- (8) Virus Webster (48), second generation, horse 6774, bled on 17/10/13.
- (9) Virus McCall (61), second generation, horse 6583, bled on 19/10/13.

Of these viruses, Nos. (3), (4), and (6) were injected, for the reasons above given, into control horses, the results being given below.

In referring to the horses inoculated with the viruses, the two letters used following the numbers of these horses refer to the groups in which these horses were inoculated in the first and second experiments respectively.

EXPERIMENT No. 3, 13TH NOVEMBER, 1913.

(1) Virus Edgar (63) 5971 (4/11/13) was injected intrajugularly, in the quantity of 10 c.c., into the five hyperimmune horses, Nos. 7077CK, 7336DM, 6764BN, 7145FK, and 7076HK.

Result.

Of these five horses, three (Nos. 7077, 6764, and 7076) showed no reactions to the inoculation. One horse (No. 7145) reacted from the 16th day to the time of the next inoculation on the 21st day, the higher temperature recorded being 103° and 104.2° on the 17th morning and evening, 105° on the 18th evening, and 104° on the 19th and 21st

evenings. Another (No. 7336) reacted to 103° on the 17th evening, and to 104° and 104.6° on the 20th and 21st evenings respectively, the latter day being that of the subsequent inoculation.

(2) Virus Dunning 6680 (5/11/13) was injected intrajugularly, in the quantity of 10 c.c., into the six hyperimmune horses, Nos. 6679CK, 7327HR, 7339DI, 7665FM, 7320HM, and 6663FO.

Result.

Of these six horses, one (No. 7320) showed no reaction. Of the others, No. 6679 showed a reaction commencing on the 12th day and still present at the time of the next inoculation on the 21st day. The higher records during this time were 102.8° , 104° , 104.8° , 106° , 104° , and 106.8° on the evenings of the 12th, 13th, 16th, 17th, 19th, and 21st days respectively; No. 7327 showed a slight tendency to react, and records of 103.6° , 103° , 103.8° , 102.8° , 102.8° , and 103° were met with on the 4th, 5th, 6th, 8th, 10th, and 14th evenings respectively; No. 7339 showed a reaction from the 1st to the 4th day, with records of 103.6° , 105° , and 104.4° on the 1st, 3rd, and 4th evenings, and again from the 8th day records of 104.2° , 104° , and 103.2° on the 5th, 10th, and 11th evenings. The death of this animal occurred on the 21st day and was then attributed to impaction of the colon. No. 7665 showed a reaction on the 3rd day, the morning and evening temperatures being respectively 104.6° and 105.6° , and the latter record was again met with on the 4th morning. The anus was relaxed on the 4th evening, and no temperature was recorded, but on the 5th evening it was again 103.8° . No further reaction was met with up to the next inoculation. No. 6663 showed a reaction to 103° on the 2nd evening, and then a reaction from the 7th day which was still present on the 21st day and lasted even beyond this latter time. The highest records between the 7th and 21st days were 104.2° , 104° , 103° , 103° , 105.4° , 103.6° , 104° , 104° , 104.8° , and 102° on the 7th, 8th, 10th, 12th, 13th, 14th, 15th, 16th, 18th, and 21st days respectively.

(3) Virus Wernick 6698 (5/11/13) was injected intrajugularly, in the quantity of 10 c.c., into the five hyperimmune horses, Nos. 7066CM, 7325DN, 6785BP, 7668FN, and 6732HL, and into a control horse, No. 8289.

Result.

The control horse, No. 8289, reacted from the 5th day and died from horse-sickness on the 8th day. Of the five hyperimmune horses, none reacted, with the exception of transient elevations to 102° and 102.4° on the 14th and 18th evenings observed in the case of No. 6732.

(4) Virus Brown 7488 (4/11/13) was injected intrajugularly, in the quantity of 10 c.c., into the five hyperimmune horses, Nos. 6911CP, 7346DP, 6675BR, 7678FN, 7939HM, and into a control horse, No. 8303.

Result.

Two of these horses (Nos. 6911 and 7939) showed no reaction. No. 6675 only showed a slight tendency to react, with evening temperatures of 102.2° and 102.6° on the 13th and 14th evenings. Another, No. 7346, showed first a reaction from the 3rd to the 5th days, with evening records of 102.6° , 104.2° , and 103° on the 3rd, 4th, and

5th days respectively, and then a reaction which, commencing on the 16th day, was still present at the time of the next injection on the 21st day, and in this reaction temperatures of 102.4° , 105° , 105° , 104° , 104° , and 104.6° were met with on the 16th, 17th, 18th, 19th, 20th, and 21st days respectively. The remaining horse, No. 7678, reacted to 103.8° and 105° on the morning and evening of the 4th day, and to 105° on the 5th morning. The anus was relaxed on the 5th evening and 6th morning, and no temperature was recorded, but the record was 102° on the 6th evening. Another slight reaction was indicated on the 13th, 14th, 15th, 16th, and 17th days by evening records of 102° , 102° , 102° , 104° , and 102° . The control horse showed no reaction up to the 21st day. (It may be noted here that this horse was then injected intrajugularly with 10 c.c. of the "mother" virus Brown 78, and this proved the susceptibility of this horse to horse-sickness by killing it with this disease on the 8th day following the inoculation.)

(5) Virus York 7228 (4/11/13) was injected intrajugularly, in the dose of 10 c.c., into the four hyperimmune horses, Nos. 6846CR, 6432BL, 7669FP, and 7326HN.

Result.

Two of these horses (Nos. 7669 and 7326) showed no reaction to the inoculation. One horse, No. 6846, showed a reaction which commenced on the 2nd day and lasted up to the 6th, with records of 103.4° , 104° , 105° , 106° , and 104.6° on the 2nd, 3rd, 4th, 5th, and 6th days; No. 6432 showed a reaction from the 7th to the 17th days, with slight remissions on the 11th, 12th, and 13th days, although the anus being relaxed on the 13th morning did not allow of the temperature being taken at this time. The higher records during the reaction were 103° , 105.4° , 104.6° , 104.8° , 104° , 104° , 104.2° , and 103° on the 7th, 8th, 9th, 10th, 14th, 15th, 16th, and 17th days respectively.

(6) Virus Martin 6766 (6/11/13) was injected intrajugularly, in the quantity of 10 c.c., into the five hyperimmune horses, Nos. 6492CL, 6479DQ, 6425BQ, 6541FQ, 6435BK, and into a control horse, No. 8400.

Result.

The control horse, No. 8400, reacted from the 4th day, and died of horse-sickness on the 6th day. Of the other horses, No. 6492 showed a reaction to 103° and 102.2° on the 4th and 5th days, and to 103.4° on the 12th day; No. 6479 showed a reaction from the 5th to the 10th days, the higher records being 102.8° , 105.8° , 105.4° , 106.2° , 104.4° , and 102.6° on the 5th, 6th, 7th, 8th, 9th, and 10th days respectively; No. 6425 showed a reaction commencing on the 1st and terminating on the 13th days, the higher records being 103.6° , 104° , 105.2° , 105.2 , 105° , 104° , 105° , 103.6° , and 103.6° on the 2nd, 4th, 5th, 6th, 8th, 9th, 10th, 11th, and 12th days respectively; No. 6541 showed an irregular reaction during the period of twenty days between this and the following injection, the higher records being 103° , 105.6° , 104° , 105° , 103° , 104° , 104.2° , and 103.8° on the 2nd, 4th, 5th, 6th, 10th, 13th, 19th, and 20th days respectively; No. 6435 reacted from the 14th to the 19th days, the higher records being 102.4° , 105° , 105.4° , 105° , and 103° on the 14th, 15th, 16th, 17th, and 18th days respectively.

(7) Virus Onderstepoort 7410 (17/10/13) was injected intrajugularly, in the quantity of 10 c.c., into the ten hyperimmune horses Nos. 7677JS, 7455JW, 7412ES, 7331IU, 7490JU, 6698GV, 6470AV, 7446IX, 6333AT, and 7156GT.

Result.

Two of these horses showed slight reactions, No. 7677 showing records of 103°, 102.4°, 103°, 102°, and 102° on the 10th, 11th, 12th, 13th, and 14th days respectively, and No. 6470 on the 5th, 6th, and 8th days recording 103°, 104.2°, 103°, and again on the 11th day 104°. No reactions were met with in the other eight horses.

(8) Virus Webster 6774 (17/10/13) was injected intrajugularly, in the quantity of 10 c.c., into the ten hyperimmune horses, Nos. 7413IS, 6328JS, 7444IW, 7488EU, 7442IU, 7257GV, 6599EV, 7318GX, 7503JT, and 5782JT.

Result.

None of these horses showed any appreciable reaction to the inoculation, with the exception of No. 7413, which showed a reaction from the 4th to the 14th days, the higher records being 102.4°, 103.2°, 105°, 105°, 105°, and 105° on the 4th, 6th, 8th, 9th, 10th, and 12th days respectively.

(9) Virus McCall (61) 6583 (19/10/13) was injected intrajugularly, in the quantity of 10 c.c., into the eight hyperimmune horses, Nos. 6766GS, 8234IS, 7293GW, 6469AU, 4177JU, 7592EV, 6601EX, and 7417IT.

Result.

Of these eight horses, one (No. 8234) showed a reaction from the 10th to the 15th days. The higher records were 102.4°, 104°, and 105° on the 10th, 11th, and 12th days respectively. From this up to the time of death, which latter occurred on the 15th day, no temperatures were recorded owing to relaxation of the anus. The death of this animal was attributed to "biliary fever." Another horse (No. 7417) showed a tendency to react indicated by an irregular temperature between the 4th and 14th days, the higher records being 104.4°, 102.4°, 102°, 102.4°, 102°, 102°, and 102° on the 4th, 6th, 7th, 8th, 9th, 12th, and 14th days respectively. With the exception of these two horses, however, no reactions were observed.

EXPERIMENT No. 4.

This experiment, using the virus McCall (59) 7348 (13/11/13), and already referred to above, was, for the reasons there given, deferred until the 21st November, 1913, and was made as follows:—

Virus McCall (59) 7348 (13/11/13) was injected intrajugularly, in the quantity of 10 c.c., into the eight hyperimmune horses, Nos. 7088JY, 8131GY, 7625EY, 6418AY, 6576AZ, 6582AZ, 7664EZ, 6314AZ, and into the control horse, No. 8268.

The result in the case of the control horse may be mentioned here for the sake of convenience, and was that the animal showed no reaction and died from debility on the 15th day. Reference has been made previously to the inoculation of this virus into the control horse, No. 8371, following on the inoculation of virus 7669 McCall (59), and the results of these inoculations will be found given there.

The results obtained in the case of the other horses will be referred to later.

Discussion of Results.

When these results of experiment No. 3 were examined on the 4th December, 1913, it seemed that certain viruses, other than McCall (59) 7904, first generation, were capable of producing a type of reaction similar to that observed before in connection with this virus, and that in these new cases the incubation period might also be a long one. We adopted this view of these reactions, since otherwise the periods of incubation we would have to credit certain viruses with possessing seemed to us to be too short in considering all the evidence we had at this point. To quote examples to which these views may be applied, we may point to horses Nos. 7339, group 2; 6846, group 5; and 6425, group 6. In the case of No. 7339, it seemed more correct to assume that the reaction setting in from the 1st day following the injection with the Dunning virus 6680, was not due to this virus, but rather due to the earlier injection of virus Onderstepoort 7410, and hence we had to accept an incubation period of 18 days for this latter virus. Again, in the case of No. 6846, we thought that the reaction appearing on the 2nd day following the injection with the York virus 7228 might perhaps be more correctly assumed to be due to the previous injection with the virus McCall (59) 7669, granting this an incubation period of 19 days. In the case of the third horse mentioned, No. 6425, we also thought it more correct to assume that the reaction observed from the 1st day following the injection with the Martin virus 6766, did not appear as a result of this injection, but was rather due to the previous injection with the virus Onderstepoort 6732, thus crediting this latter virus with a period of incubation of 18 days. These reactions were as puzzling as those before mentioned in the discussion of previous results in connection with virus McCall (59) 7904, first generation, and it is to be noted that one of the viruses mentioned above as producing this type of reaction was a descendant of this last-mentioned virus. Another point occurred in connection with horse No. 7665, group 2. This horse showed a reaction from the 3rd day following injection with the Dunning virus 6680, and we were inclined to attribute this to the previous injection with the virus Webster 6774, granting this virus an incubation period of 19 days. In looking at the chart of this animal, however, we noted that it had been inoculated in the first experiment on the 10th October with virus McCall (59) 7904, first generation, and 17 days later with the virus Webster 6774, and that two days previous to this latter injection a reaction due to the McCall (59) 7904 virus appeared, the incubation period of the latter thus being 15 days. This reaction lasted up to the 8th day following the Webster injection, and in looking at this now, in connection with the reaction following the Dunning virus, we asked ourselves the question at this stage, if instead of explaining this reaction as being due to the previous injection of Webster virus 6774, we might not more correctly assume it as being a secondary reaction of the virus McCall (59) 7904 used in the first inoculation. This will indicate that even at this time we had the idea that the condition with which we had to deal was one not hitherto encountered, the type of certain of the reactions being unfamiliar to us and not fitting in with the knowledge previously

gained in carrying out experiments with horse-sickness. That we had to deal with horse-sickness in certain cases has already been amply proved by the results obtained, but we began to suspect at this stage the presence of some other disease-producing factor, in addition to that of the virus of horse-sickness. In the case of horse No. 6541, group 6, the views expressed in connection with No. 7665 might also perhaps, though with more doubt, be applied, since this horse had been inoculated in the first experiment with virus McCall (59) 7904 on the 10th October, and 17 days later with virus Onderstepoort 6732, and then in the above experiment with virus Dunning 6680, and had shown a reaction commencing 13 days after the first injection and lasting up to the 12th day after the second injection, which latter was made during this reaction. The reaction following the last injection with the virus Dunning 6680 appeared from the 2nd day onwards, and we might, as said before, regard this reaction in the same way as that occurring in No. 7665, were it not that the second virus, Onderstepoort 6732, was itself already believed, for the reasons just given, to be capable of producing the type of reaction produced by McCall (59) 7904, first generation, whereas there was no reason to suspect the second virus, Webster 6774, used in horse No. 7665, to be capable of so doing.

We also at this stage noted certain points in connection with some of the control horses, before referred to, but which may be recalled here. Thus, the control horse, No. 8306, inoculated with the virus Onderstepoort 6732 on the 28th October, and now on the 37th day had reacted as before mentioned, but had not died of horse-sickness, and hence now on the 4th December we decided to test its susceptibility by testing it with the virus Onderstepoort 7410. As already stated, this virus produced death from horse-sickness on the 11th day following the injection. The other control horse, No. 8366, inoculated with virus Onderstepoort 6732 on the 15th November, and now on the 19th day had not died, but had recovered from a reaction observed on the 15th, 16th, and 17th days when evening temperatures of 102° , 105.2° , and 103.8° were noted, and we decided to test its susceptibility to horse-sickness later on by injecting it with the virus Onderstepoort 7410. The result of this latter test has also been referred to before, death from horse-sickness occurring on the 10th day following the injection. The control horse, No. 8414, inoculated with virus McCall (59) 7669, and now on the 37th day following this injection had shown no reaction, and so we decided to test its susceptibility by inoculation with the "mother" virus McCall (59) 7904. This, as already stated, later produced death from horse-sickness on the 10th day following this injection. The control horse, No. 8303, inoculated on the 14th November with virus Brown 7488, and not reacting to this injection up to the 20th day, we decided to test with the "mother" virus Brown 78. This, as already stated, produced a reaction, and death from horse-sickness on the 8th day following this injection.

We also wished to clear up the point further as to whether or not the blood of certain of the horses undergoing reactions in the previous experiments Nos. 1, 2, and 3, and which had not been yet tested, were really capable of producing horse-sickness, and so we inoculated the following control horses. The results of these injections are here given, as they are interesting in proving that during certain reactions

the blood of the animals undergoing these reactions contained the virus of horse-sickness:—

Control Horse.	Inoculated with Virus.	Bled on the	Experiment No.	Group.	Death, of Horse-sickness, on the
No. 8229	No. 7446	4/11/13	2	X	8th day.
No. 8413	No. 7413	23/11/13	3	8	9th "
No. 8418	No. 7325	19/10/13	1	D	11th "
No. 8228	No. 6479	19/11/13	3	6	12th "
No. 8227	No. 7444	5/11/13	2	W	9th "
No. 8211	No. 7677	26/11/13	3	7	8th "
No. 8411	No. 6432	23/11/13	3	5	—
No. 8217	No. 7331	4/11/13	2	U	—

The horse, No. 8411, showed no reaction up to the 18th day, when it died from internal hæmorrhage as the result of an accident. The horse No. 8217 showed a reaction from the 5th to the 11th days, the highest records being 103°, 103°, and 104° on the 5th, 6th, and 8th days respectively, but did not die from horse-sickness as a result of the injection. (Compare result obtained in control horse No. 8303 with virus Brown 7488.)

We decided, however, that in the next experiment we should further study the action of the three viruses,

(1) Dunning (55), second generation, horse 6680, bled on 5/11/13,

(2) Martin (52), second generation, horse 6766, bled on 6/11/13,

(3) Onderstepoort (6925), first generation, horse 7410, bled on 17/10/13, by injecting them into the horses already used in the experiments Nos. 1, 2, and 3, but selecting for use in the case of each virus horses which had not previously received an injection of the same virus. This injection was made into the following horses, the two letters following each horse indicating the lettered groups in which it was injected in the first and second experiment respectively, and the number following the two letters indicates the numbered group in which the animal was included in the third experiment.

EXPERIMENT No. 5.

Virus Dunning 6680 (5/11/13) was injected intrajugularly, in the quantity of 10 c.c., into the eighteen hyperimmune horses, Nos. 6541 FQ6, 6425 BQ6, 7677 JS7, 7412 ES7, 7490 JU7, 7331 IU7, 7446 IX7, 6333 AT7, 6470 AV7, 7156 GT7, 6698 GV7, 6435 BK6, 6492 CL6, 6432 BL5, 7488 EU8, 7442 IU8, 7257 GV8, and 6599 EV8.

Virus Martin 6766 (6/11/13) was injected intrajugularly, in the quantity of 10 c.c., into the eighteen hyperimmune horses, Nos. 7346 DP4, 7326 HN5, 7076 HK1, 7939 HM4, 6732 HL3, 6707 HO, 6785 BP3, 6911 CP4, 7669 FP5, 7444 IW8, 7293 GW9, 7320 HM2, 7327 HR2, 7455 JW7, 7318 GX8, 7503 JT8, 5782 JT8, and 6469 AU9.

Virus Onderstepoort 7410 (17/10/13) was injected intrajugularly, in the quantity of 10 c.c., into the twenty hyperimmune horses, Nos. 6328 JS8, 7413 IS8, 6766 GS9, 7665 FM2, 6679 CK2, 7077 CK1, 7336 DM1, 6764 BN1, 7145 FK1, 7066 CM3, 7325 DN3, 7668 FN3, 6675 BR4, 7678 FN4, 6846 CR5, 4177 JU9, 7592 EV9, 6601 EX9, 7417 IT9, and 6594 DO.

Result.

In discussing the results of this last inoculation it is perhaps best to refer in some slight detail to the subsequent records of certain of the above animals, in order to note in a general manner the behaviour of and reactions shown by some of these inoculated horses, and later refer briefly to the individual animals concerned.

Thus in the first group, that is to say, the horses inoculated with virus Dunning 6680, the following horses may be specially mentioned :

No. 6541. Previously injected with viruses McCall (59) 7904, Onderstepoort 6732, and Martin (52) 6766, now showed a reaction on the 15th evening to 105.4° , and on the 26th, 27th, and 28th evenings to 105° , 105° , and 104.8° respectively, again reacting to 104° on each of the 40th, 42nd, and 43rd evenings. This animal was killed on the 44th evening, the post-mortem diagnosis recorded being pernicious anaemia.

No. 6425. Previously injected with viruses Webster (48) 7764, Onderstepoort 6732, and Martin (52) 6766, now showed a reaction from the second day following the last injection, a reaction which may be said to have lasted up to the time of the animal's death on the 27th day. The higher records during this period were as follows: 104.2° on the 3rd evening, 105° on the 4th evening, 104.6° , 104° , and 104.2° on the 9th, 10th, and 11th evenings respectively, and, again, 103.4° , 104.2° , 103° , and 103° on the 14th, 18th, 20th, and 22nd evenings respectively. The death of this animal occurred on the 27th day, and lesions resembling those met with in "biliary fever" were encountered post-mortem. In the light of our present knowledge, however, and the previous history of the case, and blood examination having failed to reveal the presence of parasites, it would now seem more correct to attribute the death of this animal to the disease which we here describe.

No. 7677. Previously injected with viruses Bulawayo 7245, Martin (52) 7862, and Onderstepoort 7410, now reacted to 103.2° on the 3rd evening, to 103° , 103.2° , and 103° on the 13th, 14th, and 18th evenings respectively, and a reaction setting in from this latter date with higher records of 104.4° , 105° , 105.4° , 105° , 105.6° , and 105° on the 19th, 20th, 21st, 22nd, 23rd, and 25th evenings respectively, terminated in the death of the animal on the 26th day. The death of this animal was attributed on post-mortem examination to pernicious anaemia.

No. 7412. Previously injected with viruses Snyman 72, Martin (52) 7862, and Onderstepoort 7410, now showed a reaction of an irregular type between the 14th and 28th days, the higher evening records being 103.2° , 104° , 105° , 104° , 104° , 103.8° , 103.8° , and 103° on the 17th, 22nd, 23rd, 24th, 25th, 26th, 27th, and 28th days respectively. Another reaction to 103° , 104° , and 104.4° was observed on the 42nd, 43rd, and 45th days. From this time onwards, however, the temperature of this animal rarely exceeded 102° , excepting on the 64th evening 102.4° , 70th evening 102.4° , 85th and 92nd evenings 103° and 102.6° respectively; and a slight reaction from the 124th to the 128th days, the evening records of the 124th and 126th days being 103° . This animal eventually died on the 180th day following the injection as a result of debility.

No. 7490. Previously injected with viruses Bulawayo 7245, Brown 78, and Onderstepoort 7410, now showed a reaction with the maximum records of 105.2° , 104° , 103.2° , 103.2° , 104.6° , 103.6° , and 104° on the 16th, 17th, 22nd, 23rd, 24th, 26th, and 27th evenings respectively; again reacting to 103° , 104° , and 103° on the 45th, 46th, and 47th evenings; to 102.4° and 102.6° on the 87th and 88th evenings, and to 103° on the 97th evening. Following this, however, and up to the 140th day, the highest records were 102° , 102° , and 102.4° on the 106th, 117th, and 133rd evenings. A slight reaction to 103° , 102° , 103° , and 102° was observable on the 140th, 141st, 142nd, 143rd days respectively, but from this time onwards to the 186th day the temperature never exceeded 101° . On the 186th day, however, a slight reaction occurred, the temperatures recorded being 101.2° , 104.2° , 103° , 103.2° , 103.8° , and 100.4° on the 186th morning, 186th evening, 187th morning, 187th evening, 188th morning, and 188th evening respectively, and a rise of temperature to 102° on the morning and evening of the 190th day was also noted. From this time onwards the temperature never reached 102° up to the 231st day. On this latter day, however, the temperature rose to 103° in the morning, registered 104.4° in the evening, and from this time up to the time of death on the 241st day the following temperatures were recorded: 232nd day, morning 102.8° , evening 101° ; 233rd day, morning 104.2° , evening 101.4° ; 234th day, morning 101.6° , evening 103° ; 235th day, morning 102° , evening 102° ; 236th day, morning 101° , evening 102.4° ; 237th day, morning 102° , evening 101° ; 238th day, morning 100° , evening 100° ; 239th day, morning 99° , evening 103.4° ; 240th day, morning 103.4° , evening 105° ; and 241st day, morning 98.6° . On the 241st morning this animal was down and unable to rise, and its death occurring on this day was attributed to pernicious anaemia.

Two other cases in this group which may be described in a similar way are horses 7331 and 7446.

No. 7331. Previously injected with viruses Edgar (88) 8200, Brown 78, and Onderstepoort 7410, as previously mentioned, now showed a reaction to 103° on the 14th evening, to 104.2° and 105.2° on the 15th morning and 15th evening respectively, and to 103° on the 17th day. A reaction then occurred from the 21st to the 27th days with records of 102.8° , 104.6° , 104.8° , 104.6° , 104° , and 103° on the 21st, 22nd, 23rd, 25th, 26th, and 27th evenings respectively, then a remission up to the 30th occurred, a further reaction then setting in, and this with records of 103.8° , 103.6° , 104.4° , 105° , 105° , 104.6° , 105° , and 103° on the 31st evening, 32nd morning, 32nd evening, 33rd morning, 33rd evening, 34th morning, 34th evening, and 35th morning, terminated on this latter day in a remission which lasted up to the 42nd day. On the evening of this day the temperature was 103.6° , and on the following day the morning and evening records were respectively 106.4° and 105.2° ; on the 44th day 102.2° and 101° ; on the 45th day, on which day the animal was down and unable to rise, and when the animal died, the records of the morning and evening respectively were 100.4° and 102.8° . This animal, as just mentioned, died on the 45th day, the cause of death being attributed to pernicious anaemia.

No. 7446. Previously injected with viruses Edgar (88) 8200, Edgar (63) 7937, and Onderstepoort 7410, and having behaved as previously stated, now showed a reaction from the 14th day, which terminated in the death of this animal on the 26th day. The complete

temperature reaction of this animal during this time may here be given from the 14th day following the injection (18th December). This reaction was as follows:—

14th Day.....	Morning,	101.4°	Evening,	104.8°
15th ..	"	104.4°	"	105.4°
16th ..	"	103.6°	"	104°
17th ..	"	101.6°	"	100.2°
18th ..	"	100°	"	102.4°
19th ..	"	102.4°	"	103.6°
20th ..	"	104°	"	106°
21st ..	"	105°	"	105°
22nd ..	"	102.6°	"	105.6°
23rd ..	"	105.2°	"	105.2°
24th ..	"	103.8°	"	102.4°
25th ..	"	104°	"	104°
26th ..	"	102.6°	"	103.6°

The death of this animal from pernicious anaemia occurred on the 26th day.

Having described in some detail these foregoing seven cases, other cases of this group may be more briefly discussed. Thus *horse* 6333 showed a reaction from the 14th day up to the 40th day, and on this latter day was killed on account of being down and unable to rise, the lesions found on post-mortem examination being ascribed to pernicious anaemia.

Horse 6492 showed a reaction from the 14th day which lasted up to the time of death of this animal from pernicious anaemia on the 20th day with the exception of a remission on the 16th and 17th days.

Horse 7488 showed a reaction on the 17th and 18th days to 105.2° and 106° on the evenings of those days, the temperature again rising to 102.8°, 103.8°, 103.8°, and 102.8° on the 24th, 25th, 26th, and 27th days. A remission then occurred up to the 35th day, when a further reaction set in, and finally the death of the animal from pernicious anaemia occurred on the 48th day.

Horse 7442 also showed a reaction following this injection, which, commencing on the 12th day, lasted up to the 24th day with a slight remission on the 18th and 19th days, and again showed an irregular reaction from the 34th day up to the time of death from pernicious anaemia on the 48th day. A remission was noted on the 39th, 40th, and 41st days, and on the 36th, 37th, 43rd, and 44th days the relaxed condition of the anus did not allow of temperatures being recorded.

Horse 7257 showed first a reaction from the 14th to the 18th days, then a remission to the 22nd day, when a reaction lasting up to the 27th day set in, a further remission to the 30th day, and, finally, a reaction from the 31st day, which, with maximum records of 104.6°, 105°, 105.6°, and 104° on the 31st, 32nd, 35th, and 36th days respectively, lasted up to the 39th day, when the animal died, the cause of death being attributed to pernicious anaemia.

Horse 6470 also showed several reactions following this injection, the first occurring between the 12th and 15th days, the evening records of the 12th, 13th, 14th, and 15th days being 103.8°, 103.8°, 103.4°, and 104.2° respectively. A remission lasting up to the 29th day was then noted, but from the 30th to the 33rd days a reaction with records of 107°, 105.4°, 104.4°, and 104° on the evenings of the 30th, 31st,

and 32nd days, and morning of the 33rd day occurred. A further remission up to the 46th day was then noted, but from this latter day a reaction set in which lasted up to the time of death of this animal on the 57th day from pernicious anaemia. During this last reaction the highest records were 106° , 106.4° on the 47th day, morning and evening respectively, 105° and 104.4° on the 48th day, 104.4° on the 50th day, and 104° and 105° on the morning of the 53rd and evening of the 54th day respectively. Further, the temperature during this reaction only descended below 103° on the 49th, 53rd, 55th, and 56th days, and in the evenings the lowest temperature recorded on those occasions being 102.4° .

Horse 6698 also showed a reaction from the 14th to the 17th days, and again from the 25th day up to the time of death from pernicious anaemia on the 39th day.

Horse 7156 also reacted following the injection, and between the 12th and 20th days, but the death of this animal, caused by quinine poisoning, occurred on the 42nd day.

In referring to results obtained following injection with the viruses Martin (52) 6766 and Onderstepoort 7410, we may compare them with the cases described above, and state that in the case of certain horses inoculated with these latter viruses reactions similar to those we have just described were also noted.

Thus horse 7326 of the Martin group may be noted as showing a well-marked reaction from the 13th to the 22nd day, with higher records of 103.8° , 103.6° , 103.6° , 104° , 104° , 104.2° , and 104° on the 13th, 14th, 15th, 16th, 17th, 21st, and 22nd days respectively, and again a reaction from the 104th to 108th days, with records of 104.6° and 105.6° on the evenings of the 105th and 106th days. A later reaction was observed on the 165th and 166th days, with evening records of 104.8° and 103° on each of these days respectively.

In the group inoculated with virus Onderstepoort 7410, horse 7592 may similarly be noted as a horse showing repeated reactions following inoculation with this virus. This horse, which had not reacted following any of the previous injections, now showed, first a reaction between the 12th and 24th days following the injection with virus Onderstepoort 7410, then a well-marked reaction from the 33rd to the 36th days, another between the 43rd and 47th days, and again one from the 65th to the 69th days, and, later, one appearing from the 87th to the 98th days. On the 169th and 170th days a reaction with maximum records of 103.8° and 104° was noted, and between the 194th and 198th days another reaction with maximum records of 104° , 103° , 103° , and 103.2° on the 194th, 195th, 196th, and 198th days occurred. From this time onwards, however, and up to the time when the animal was killed on the 254th day, the temperature did not exceed 102° .

This condensed examination of the results noted following the inoculation of the three viruses, Dunning 6680 (55), second generation, Martin 6766 (52), second generation, and Onderstepoort 7410, first generation, will thus serve to show the general nature of the reactions encountered, but it should be noted that reactions were not observed in the case of every horse injected.

Reviewing the results of all of these inoculations and the temperature charts of all these animals, at the present time it would appear that only certain viruses were capable of producing the particular type

of reactions we have especially referred to in the preceding record of results, or, in other words, were capable of producing the disease we describe. Although, however, certain viruses can apparently be excluded from blame in this respect, yet there are certain other viruses which, though they cannot be excluded in this fashion, still remain in a rather doubtful position.

To make this point clearer, we may now give a list of the horses inoculated in the last experiment, referring to them under the heading of the particular virus with which they were inoculated in the last experiment, thus considering them under the headings of virus Dunning 6680, virus Martin 6766, and virus Onderstepoort 7410, and in this list the virus which seemed to be that which produced the reactions indicating the presence of the disease described is indicated by a double line under the virus believed to be the infecting one. The viruses opposite the number of each animal are the viruses with which the animal was successively inoculated with in the first, second, and third inoculations respectively, and where the double line is placed under the number of the animal itself, the last injected virus (under which heading the animal is dealt with) is assumed to be the virus producing the disease. Single lines placed under a virus indicate reactions occurring between the time of injection of that virus and the subsequent inoculation with another virus, and in those cases where double lines are placed underneath two different viruses with which the same animal was injected it is intended to indicate that it is uncertain as to which of the two viruses actually produced the disease.

Group inoculated with Dunning virus 6680 (55), second generation.

Horse

6541 McCall (59) 7904, Onderstepoort 6732, Martin 6766.

Died of pernicious anaemia on the 44th day after last injection.

6425 Webster 7764, Onderstepoort 6732, Martin 6766.

Died of "biliary fever"? on the 27th day after last injection.

7677 Bulawayo 7245, Martin 7862, Onderstepoort 7410.

Died of pernicious anaemia on the 26th day after last injection.

7412 Snyman 72, Martin 7862, Onderstepoort 7410.

Died as result of debility on the 180th day after last injection.

7490 Bulawayo 7245, Brown 78, Onderstepoort 7410.

Died of pernicious anaemia on the 241st day after last injection.

7331 Edgar 8200, Brown 78, Onderstepoort 7410.

Died of pernicious anaemia on the 45th day after last injection.

7446 Edgar 8200, Edgar 7937, Onderstepoort 7410.

Died of pernicious anaemia on the 26th day after last injection.

Horse

6333 Ziervogel 7483, York 7950, Onderstepoort 7410.

Killed (pernicious anaemia) on 40th day after last injection.

6470 Ziervogel 7483, Wernick 8096, Onderstepoort 7410.

Died of pernicious anaemia on 57th day after last injection.

7156 Steenekamp 73, York 7950, Onderstepoort 7410.

Reacted following last injection, but died from quinine poisoning on the 42nd day at time when a second reaction had commenced. Poisoning occurred as result of the large dose of the drug given in attempting to treat this reaction.

6698 Steenekamp 73, Wernick 8096, Onderstepoort 7410.

Died of pernicious anaemia on the 39th day after last injection.

6435 Webster 7764, Rickertsdam 7336, Martin 6766.

This horse reacted definitely and repeatedly, and later appeared clinically to recover, but its blood was still virulent when tested on the 30th June, 1914, and 20 c.c. of its blood then injected intrajugularly into horse 8443 caused the death of this animal on the 33rd day from pernicious anaemia.

6492 McCall (61), 7926, Rickertsdam 6479, Martin 6766.

Died of pernicious anaemia on 20th day after last injection.

6432 Webster 7664, Rickertsdam 6479, York 7228.

Reacted typically following last injection, but later, accidentally contracting glanders, died of this disease on the 162nd day after the last injection.

7488 Snyman 72, Brown 78, Webster 6774.

Died of pernicious anaemia on 48th day after last injection.

7442 Edgar 8200, Brown 78, Webster 6774.

Died of pernicious anaemia on 47th day after last injection.

7257 Steenekamp 73, Wernick 8096, Webster 6774.

Died of pernicious anaemia on 39th day after last injection.

6599 Snyman 72, Wernick 8096, Webster 6774.

This horse was killed on account of poor condition and debility on the 228th day following last injection.

Group inoculated with Martin virus (52) 6766, second generation.

7346 Rickertsdam 7966, Onderstepoort 7410, Brown 7488.

This animal died on the 43rd day following last injection. Hæmoglobinuria was present on post-mortem examination, but protozoan parasites had not been seen in the blood examined during the reaction and could not be detected in the spleen smears made just after death. The cause of the hæmoglobinuria, though remaining

undetermined, was therefore not attributed to Nuttalliosis, and death is regarded as due to pernicious anaemia, with hæmoglobinuria occurring as the result of some complication.

Horse

7326 Onderstepoort 6025, McCall (61) 6679, York 7228.

Reacted following last injection as before referred to, and was finally discharged from experiment on the 260th day after last injection.

7076 Onderstepoort 6025, Rickertsdam 7336, Edgar 5971.

This horse, not reacting, was later used as a "control" for injection of blood taken from horses reacting to Dunning virus 6680 on the 29/12/13, and following their injection with this latter virus on the 4/12/13. It contracted pernicious anaemia and died of this disease on the 217th day following this last injection.

7939 Onderstepoort 6025, Webster 6774, Brown 7488.

This animal did not show reactions to any of these injections and later died on the 143rd day following the last injection. Pulmonary stasis was the only markedly pathological condition found on post-mortem.

6732 Onderstepoort 6025, Rickertsdam 6479, Wernick 6698.

Doubtfully reacted following last injection and was discharged from experiment on the 260th day following last injection.

6707 Onderstepoort 6025, McCall (61) 6583.

Reacted slightly following last injection and was finally discharged from experiment on 260th day following last injection.

6785 Webster 7764, Onderstepoort 7410, Wernick 6698.

Showed repeated slight reactions following last injection, and was killed on account of debilitated condition on the 171st day.

6911 McCall (61) 7926, Onderstepoort 7410, Brown 7488.

Showed no reaction to any of these injections, and, later, accidentally contracting glanders, died from this disease on the 93rd day following last injection.

7669 McCall (59) 7904, Onderstepoort 7410, York 7228.

This horse accidentally contracted glanders and died from this disease on the 71st day following last injection.

7444 Edgar 8200, Dunning 7872, Webster 6774.

Reacted typically and repeatedly following last injection, and was eventually killed on account of poor condition on the 206th day following last injection.

7293 Steenekamp 73, Dunning 7872, McCall (61) 6583.

Reacted typically and repeatedly following last injection, but later accidentally contracted glanders, and died from this disease on the 157th day following the last injection.

Horse

7320 Onderstepoort 6025, Webster 6774, Dunning 6680.

Shown a well-marked reaction from the 17th to the 37th days following last injection, i.e. lasting 20 days, and steadily lost condition, but showed no further well-marked reaction up to time when killed on account of debility on the 215th day following last injection.

7327 Onderstepoort 6025, McCall (59) 7669, Dunning 6680.

Shown a reaction from the 6th to the 11th days following last injection, doubtfully showing later slight reactions, and was finally discharged from experiment on the 260th day following last injection.

7455 Bulawayo 7245, Dunning 7872, Onderstepoort 7410.

Reacted following injection with Dunning virus 7872 with "dikkop" symptoms, but did not react further, and was finally discharged from experiment on the 260th day following the last injection.

7318 Steenekamp 73, Edgar 7937, Webster 6774.

Did not react to these injections, but accidentally contracted glanders, and died of this disease on the 158th day following the last injection.

7503 Bulawayo 7245, York 7950, Webster 6774.

This horse did not react to these injections, but was used on the 29/12/13 as a "control" animal for injection with blood of horses reacting following injection with virus Onderstepoort 7410 on 4/12/13. It may be noted that it contracted pernicious anaemia from the 6th to the 23rd days following this injection, and on the last-mentioned day was killed, being then down and unable to rise.

5782 Bulawayo 7245, York 7950, Webster 6774.

The first well-marked reaction commenced on the 30th day following the last injection, and death from pernicious anaemia occurred on the 94th day.

6469 Ziervogel 7483, Brown 78, McCall (61) 6583.

This horse did not react to any of these injections, and was discharged from experiment on the 260th day following the last injection.

Group Inoculated with Onderstepoort 7410.

6328 Bulawayo 7245, Martin 7862, Webster 6774.

Shown no reaction following third or fourth injection, and died of gastric impaction on the 74th day following last injection.

7413 Edgar 8200, Martin 7862, Webster 6774.

Reacted typically and repeatedly following last injection, and was finally killed for pathological examination on the 166th day following the last injection.

Horse

6766 Steenekamp 73, Martin 7862, McCall (61) 6583.

Reacted typically and repeatedly following last injection, reactions being observable as late as 168th to 171st day, and again on 226th and 227th days. Finally discharged from experiment on 260th day following last injection. Reacted following Martin virus with slight "dikkop" symptoms, and the pernicious anaemia reactions following last injection seem more probably caused by the last inoculation.

7665 McCall (59) 7904, Webster 6774, Dunning 6680.

This horse, though reacting up to the time of the fourth inoculation, did not react subsequent to this injection, and died from "poverty" on 156th day following this last injection.

6679 McCall (61) 7926, Rickertsdam 7336, Dunning 6680.

This horse died on the 14th day following the last injection. A reaction lasting up to time of the animal's death was in progress at time of fourth injection, and was apparently produced by the virus Dunning 6680. The cause of death of this horse was attributed on 18/12/13 to biliary fever, but in the light of present knowledge appears most probably to have been also due to pernicious anaemia.

7077 McCall (61) 7926, Rickertsdam 7336, Edgar 5971.

Reacted typically and repeatedly following the last injection, but later accidentally contracting glanders, died from this disease on the 180th day following the last injection.

7336 Rickertsdam 7966, Webster 6774, Edgar 5971.

Reacted typically and markedly following last injection and died from pernicious anaemia on the 39th day following the last injection.

6764 Webster 7764, McCall (61) 6679, Edgar 5971.

Showed a well-marked reaction from 15th to 19th days following last injection, and slightly reacting later even up to 96th and 100th day, eventually died in poor and anaemic condition on 135th day following the last injection.

7145 McCall (59) 7904, Rickertsdam 7336, Edgar 5971.

Died from pernicious anaemia on 56th day following last injection, having reacted typically and repeatedly following injection with virus McCall 7904. A fibrinous peritonitis was found on post-mortem to be present in addition to the lesions more usually met with.

7066 McCall (61) 7926, Webster 6774, Wernick 6698.

This horse did not react to any of the injections, but later contracted Nuttalliosis, dying from this disease on the 58th day following the last injection.

Horse

- 7325 Rickertsdam 7966, McCall (61) 6679, Wernick 6698.
 Reacted typically from the 14th day following last injection, and died on the 29th day following this same injection.
- 7668 McCall (59) 7904, McCall (61) 6679, Wernick 6698.
 This horse showed no definite reaction following any of the above inoculations, and was eventually discharged from experiment on the 260th day following the last injection.
- 6675 Webster 7764, McCall (59) 7669, Brown 7488.
 Reacted with "dikkop" symptoms to the first injection, but did not react subsequently, and was finally killed on the 36th day following the last injection.
- 7678 McCall (59) 7904, McCall (61) 6679, Brown 7488.
 Reacted typically and repeatedly from time of injection with virus McCall 7904, but was one of the horses accidentally contracting glanders during an outbreak of this disease, and death from this cause occurred on the 170th day following last injection.
- 6846 McCall (61) 7926, McCall (59) 7669, York 7228.
 The reaction of this horse to virus McCall 7669 in experiment No. 2 has already been referred to in discussing results of experiment No. 3. Only one definite reaction, that from the 38th to 41st days, was observable subsequent to the last injection, and the animal was eventually discharged from experiment on the 260th day following the last injection.
- 4177 Bulawayo 7245, Brown 78, McCall (61) 6583.
 Never showed any reaction to any of these injections and maintained a temperature curve showing remarkably little variation up to the time of discharge from experiment on the 260th day following last injection.
- 7592 Snyman 72, Wernick 8096, McCall (61) 6583.
 Reacted typically and repeatedly, even up to 200 days, after the last injection, and was eventually killed on the 254th day for pathological examination.
- 6601 Snyman 72, Edgar 7937, McCall (61) 6583.
 This horse did not react to any of these injections, and was subsequently discharged from experiment on the 260th day following last injection.
- 7417 Edgar 8200, York 7950, McCall (61) 6583.
 This horse did not react to any of the above injections, and was then used as a "control" for injection with blood of five horses, which, having been inoculated with virus Martin 6766 on the 4/12/13, were reacting subsequently on the 29/12/13. Slight reactions were observed subsequent to this latter injection, and this animal was eventually discharged from experiment on the 235th day from the time of the last injection.

Horse

6594 Rickertsdam 7966, McCall (61) 6583.

Reacted typically and repeatedly following injection No. 2, but the reaction was apparently the result of injection No. 1. Continued to react markedly and repeatedly following the last injection, and died of pernicious anaemia on the 149th day following the last injection and on the 204th day from the time of the first injection.

This list of results can thus be seen to bring out a number of points, and it will be seen from it that there is no reason to suspect, as causing the disease, the following viruses: Ziervogel 7483, Webster 7664, 6774, McCall (61) 7926, 6679, 6583, Snyman 72, Steenekamp 73, Edgar (80) 8200, Bulawayo 7245, York 7950, 7228, Wernick 8096, 6698, Edgar (63) 7937, 5971, Mule Inspector O. W. 75, and Onderstepoort 7781.

On the other hand, and excluding these viruses just mentioned from suspicion in this respect, there are the viruses McCall (59) 7904, 7669, Martin 6766, Dunning 6680, in one group; the viruses Onderstepoort 7410, Onderstepoort 6732, and Rickertsdam 7966 in another group; and the viruses Rickertsdam 6479, Brown 7488, and Martin 7862 in a third group, all of which appeared capable of producing the disease.

This behaviour is well marked in the case of the first of the three groups of viruses mentioned immediately above, also to be noted in those of the second group, but not so markedly, and not with at all so much certainty in those of the third group.

Horses which may be referred to in connection with this point and in reference to each of these viruses are—

- virus McCall (59) 7904, horses 6541, 7665, 7145, 7678, 6663;
- virus McCall 7669, horse 6846.
- virus Martin 6766, horses 6435, 7326, 6707, 6785, 7444, 7293, 7320, and 5782;
- virus Dunning 6680, horses 7677, 7412, 7490, 7331, 7446, 6333, 7156, 6698, 7488, 7442, 7257, 6599, 6679, (?6470 and ?6492);
- virus Onderstepoort 7410, horses 7325, 7592 (and ?6470, ?7346, ?7413, ?6766, ?7336);
- virus Onderstepoort 6732, horse 6425;
- virus Rickertsdam 7966, horse 6594 and ?7336;
- virus Rickertsdam 6479, horse ?6432;
- virus Brown 7488, horse ?7346;
- virus Martin 7862, horses ?7413 and ?6676.

If this explanation is accepted, however, then it would appear necessary to assume different degrees of virulency for at least some of these viruses. Thus virus Onderstepoort 7410 might be referred to as apparently not producing reactions in a number of animals into which it was injected. It must be admitted, however, that where inoculation by this virus was subsequently followed by injection of another virus capable of producing the disease that the reaction following this latter injection may have masked a reaction due to the previous injection with virus Onderstepoort 7410, that is to say, that two reactions may have been overlapping, as it were, or the one reaction noted may have represented a combined effect of the two viruses used.

This point appears in a number of cases in the horses injected in the last experiment with virus Dunning 6680, and where the disease appears to have followed this injection, but where virus Onderstepoort 7410 had been used in the immediately preceding injection. That the "individuality" or "individual resistance" of the injected animal is also a factor which has, however, to be reckoned with in considering these results seems to be established by certain horses such as No. 7076, which though not reacting to virus Martin 6766 (10 c.c. intrajugularly), yet, later, contracted the disease when injected with a mixture and larger quantity (120 c.c. intrajugularly) of viruses taken on the 29/12/13 from horses reacting subsequent to inoculation with virus Dunning 6680 on the 4/12/13. Horse 7668 is another animal which might be referred to in this respect, for although receiving virus McCall (59) 7904 as a first inoculation and virus Martin 6766 as a fourth injection, receiving two non-suspected viruses between these injections, yet it never showed any reaction at all and was discharged from experiment on the 260th day following the last injection.

The curious behaviour of certain of the control horses already referred to in connection with their failure to react when injected with virus Onderstepoort 6732 and McCall 7669 and 7348, is a point to which reference may again be made, although difficult to explain, and Onderstepoort 6732, when later injected into two control horses, Nos. 8458 and 8464, on the 20/12/13, and in the dose of 10 c.c. intrajugularly, also failed to produce definite symptoms of the disease. Similar behaviour on the part of virus McCall 7348 was noted when injected into a control horse, 8420, on the 9/12/13 and 8/1/14, in the doses of 10 c.c. and 100 c.c. intrajugularly respectively.

That the virus 7348 McCall (59) was, however, in itself capable of causing the disease is undoubtable in considering the result of experiment No. 4, which we have already referred to as being made on 21st November, 1913 (details of injection also before referred to, see experiment No. 4), and in which experiment eight horses were inoculated with this virus in the quantity of 10 c.c. each intrajugularly.

The results of this experiment may be mentioned now in stating that horse 7088 with an incubation period of 10 days to the first reaction later reacted typically and repeatedly, a well-marked reaction (with maximum temperature of 105.6° and 105° on the 146th and 147th days) being apparent even from the 144th to the 148th days following the injection, and this animal was finally discharged from experiment on the 267th day; horse 8131 also contracted the disease and died from pernicious anaemia on the 123rd day; horse 7625 showed typical and repeated reactions, the last well-marked reaction being noted between the 187th and 192nd days, and was discharged from experiment on the 273rd day; horses 6418 and 6582 contracted the disease and died on the 33rd and 49th days respectively following the injection; horse 7664 showed repeated but not well-marked reactions following the injection, and was discharged from experiment on the 273rd day; horse 6314 also reacted and with an incubation period of twelve days, death occurring on the 20th day; horse 6576 also reacted typically and repeatedly, and, later, appeared to be clinically recovered, but that it remained a "virus-carrier" or "virus-reservoir" despite this *apparent* recovery is proved by the fact that the injection of its blood, in the quantity of 20 c.c., given intrajugularly to horse 7785 on the 30th June, 1914 (i.e. 221 days following

the inoculation), caused this latter horse to contract the disease from which it later died.

These observations here referred to, along with the fact that during the third week in December, 1913, examination of the blood of the horses inoculated with the viruses Dunning 6680, Martin 6766, and Onderstepoort 7410 and then undergoing reactions failed to show the presence of parasites in the blood on microscopical examination, were thus those which led us towards this time to believe that we had to deal, neither with Nuttalliosis nor a modified form of horse-sickness, but with a disease not hitherto recognized as existent in South Africa, and the similarity of the clinical symptoms of the disease to those described in equines in other countries affected with the disease variously known as equine pernicious anaemia, infectious equine anaemia, swamp fever, etc., led us to think of the possibility that the disease with which we had to deal was similar or identical with that just referred to. The opinion that this was so, was strengthened in considering again the few post-mortem examinations we had had the opportunity of carrying out at that time, and also by the post-mortem of horse 6418 made on the 25th December, 1913.

This view then led to the inoculation on the 29th December, 1913, of certain control horses with mixtures of blood taken from horses reacting subsequent to the injection of the viruses Dunning 6680, Martin 6766, and Onderstepoort 7410 on the 4th December, 1913, and the results of the injections of these mixtures into horses 7076, 7417, and 7503 have already been referred to when discussing the results obtained from the injection of these three viruses.

Following this, attempts to transmit the disease to other animals, and experiments with filtered blood or serum, as well as experiments in which blood or urine of affected animals was administered to horses by the mouth, were made, and these will be referred to in the following pages.

The general symptomatology, post-mortem appearances, and other characters of the disease will there be also dealt with, and from these records will be seen what we believe to be our justification in regarding the disease as similar to or identical with that described in other countries such as Europe (France, Germany, Belgium, Hungary, etc.), North America, United States and Canada, and Japan, and under the various names: *Anaemia infectiosa equorum*, infectious anaemia of equines, equine pernicious anaemia, *anémie infectieuse*, *anémie pernicieuse*, *typho anémie infectieuse du cheval*, *anémie pernicieuse progressive*, *anémie epizootique*, *infektiöse Blutarmut der Pferde*, *perniziöse Anämie der Pferde*, "swamp fever," "river bottom disease," "plains paralysis," etc.

In pursuing this programme we shall first refer to our experimental attempts to transmit the disease to animals of species other than the horse.

EXPERIMENTS MADE IN ATTEMPTING TO TRANSMIT THE DISEASE TO ANIMALS OF SPECIES OTHER THAN THE HORSE.

(a) The first attempt to transmit the disease to animals other than the horse was made in an experiment carried out on the 13th January, 1914, using the blood of horse 6766.

This horse, it should be noted, has already been referred to in considering the results of experiment No. 5 in the group then inoculated with virus Onderstepoort 7410, and was a horse which showed

symptoms of the disease in a series of definite reactions which were noted for a long period, and on many occasions, subsequent to this last injection.

It was at the commencement of one of these reactions, and on the 13th January, 1914, that the blood used in this experiment was withdrawn, defibrinated, and injected as follows:—

Cattle 3057 and 3058 each received 20 c.c. subcutaneously.

Sheep 6371 and 6372 each received 10 c.c. subcutaneously.

Goats 5572 and 5573 each received 10 c.c. subcutaneously.

Result.

The result of this experiment, however, was negative, and none of the animals showed any sign of reaction up to the 21st February, 1914, i.e. during a period of 39 days following the inoculation.

(b) In the next experiment of this kind (which also yielded negative results) these same animals were again utilized, being this time, however, injected with the blood of horse No. 6594.

This horse, No. 6594 (also referred to in connection with horses 8574, 8531, 5855, 5856, mule 3278, and donkey 3712), has already been referred to in experiment No. 5 in connection with the horses in the group inoculated with virus Onderstepoort 7410, and was a horse which, reacting definitely and repeatedly following this last injection, later died from pernicious anaemia on the 149th day following its last inoculation.

On the 21st February, 1914, blood withdrawn from this horse was defibrinated and injected as follows:—

Cattle 3057 and 3058 each received 20 c.c. subcutaneously.

Sheep 6371 and 6372 each received 10 c.c. intrajugularly.

Goats 5572 and 5573 each received 10 c.c. intrajugularly.

In addition to this the two above-mentioned cattle were injected two days later (23/2/14) with the "whole" blood of the same horse freshly withdrawn, and on this occasion each of the animals was injected with 10 c.c. intrajugularly.

Result.

No reaction was noted to occur in any of these animals, although temperature records were kept up to the 7th June, 1914.

(c) In another experiment, where the blood of horse 8470 was utilized and injected into mule 3278 and donkey 3712, the results obtained were also negative.

This horse, No. 8470, had contracted the disease from the injection of a mixture of viruses obtained from horses reacting subsequent to the inoculation of virus Martin 6766 on the 4/12/13, and had been injected with this mixture on the 29/12/13.

Blood was withdrawn from this animal, defibrinated, and injected into the mule and donkey above referred to, each receiving 20 c.c. intrajugularly, on the 21/1/14, but neither of these animals showed any reaction to this injection up to the 21/2/14.

On this latter date they were therefore again inoculated, this time each receiving 20 c.c. intrajugularly of the same defibrinated blood of horse 6594, that was used on the same date for the injection of the animals referred to in the immediately preceding experiment.

Result.

No definite reaction was noted to occur in either of these animals as a result of this injection, though temperature records of both animals were kept up to the 21/8/14.

(d) All of the above experiments apparently having yielded negative results, another experiment of a similar nature was carried out on the 3rd July, 1914, the blood of horse No. 6435 being utilized on this occasion.

This horse, No. 6435, has already been referred to in considering the results of experiment No. 5, and in the group of horses inoculated with virus Dunning 6680. It reacted typically and definitely subsequent to inoculation with virus Martin 6766, and its blood, when taken on the 30th June, 1914, and injected in the quantity of 20 c.c. intrajugularly into horse 8443, caused this animal to contract the disease, from which it died on the 33rd day. Horse 6435 was not reacting on the day when the blood used for the injection of horse 8443 was withdrawn, but on the day following a well-marked reaction set in lasting from the 1st to 4th July, 1914, and it was during this reaction that the blood used in the experiment we are now dealing with was taken, the temperature of horse 6435 at this time being 105°.

Three mules, three donkey foals, two cattle, two sheep, two goats, and two dogs were inoculated with this blood, and temperature records of these animals were kept up to the 21st August, 1914, i.e. up to the 49th day following the inoculation. On this later date these animals were discharged from experiment, but before being discharged they were separately bled. These blood samples were then, in the case of animals of the same species, mixed together, equal quantities from each animal being taken, and each of the mixtures so obtained was injected into a control horse, a separate control animal being used for each "species-mixture."

The details and results of this experiment are as follows:—

Animals inoculated on 3rd July, 1914, with blood of horse 6435.

Mules 5161, 5468, 5497, each 20 c.c. blood intrajugularly.

Donkey Foals 8428, 8433, 8479, each 20 c.c. blood intrajugularly.

Cattle 3226, 3254, each 20 c.c. blood intrajugularly.

Sheep 6041, 6042, each 10 c.c. blood intrajugularly.

Goats 5599, 5600, each 10 c.c. blood intrajugularly.

Dog 1290, 10 c.c. blood intrajugularly.

Dog 1291, 10 c.c. blood subcutaneously.

Result.

In the case of the cattle, sheep, goats, and dogs used in the above experiment no reaction to this inoculation was apparent in these animals, nor did their blood, when injected as above into control horses (Nos. 8597, 8598, 8542, and 8610 respectively) and in the quantity of 60 c.c. intrajugularly of the mixtures, produce a typical reaction in any of these animals.

The behaviour of the three mules and the three donkey foals was, however, different, and although no marked reaction to the inoculation was shown by these animals, yet the mixtures of their blood taken on the 49th day, and inoculated as above stated into the control horses (Nos. 7585 and 8294), produced the disease in these latter animals.

Thus mule 5161 up to the 14th day never exceeded 101° in temperature, but on the 14th, 15th, and 16th evenings the records were 102.8°, 102.2°, and 104° respectively, and 101.8° was again recorded

on the 21st and 29th evenings. The only occasion on which the temperature again reached 102° was on the evening of the 43rd day.

In the case of mule 5468 the only notable, though slight, rise in temperature was that to 103° on the evening of the 12th day to 102° , 102.2° , and 102° on the evenings of the 18th, 19th, and 20th days respectively, and to 102° and 101.4° on the 46th and 48th evenings respectively.

Mule 5497 showed a rise of temperature to 102° on the 5th evening, but no further rise to this point was noted again up to the 31st and 32nd days, when the evening records were 102.4° and 102° respectively. Later, however, a well-marked temperature reaction was noted from the 42nd to the 45th days, with records of 103° , 103.4° , 103° , and 104° on the evenings of the 42nd, 43rd, 44th, and 45th days, the pulse rate at the same time being somewhat increased in frequency and up to 64, 68, and 76 per minute on the 45th, 46th, and 47th days, an orange-yellow coloration of the conjunctival membrane being also apparent.

Otherwise no marked clinical symptoms, save a swelling of the hind legs of mule 5161 appearing about the 17th day, were noted in the case of these animals.

The mixture of their blood, however, injected in the quantity of 60 c.c. intrajugularly into horse 7585 caused the disease to appear in this animal after an incubation period of about eleven days, and the reaction then appearing persisted up to the time of the animal's death from pernicious anaemia on the 29th day.

The swaying gait and weakness of the hindquarters met with in this disease appeared in this horse on the 17th day, and ecchymosis of the conjunctival membrane, present at first on an orange background which later assumed a dirty yellow colour, was observed—examination of the blood for parasites proving negative.

In the donkey foals the only elevations of temperature noted were as follows:—No. 8428 to 103° on the 7th and 20th evenings, to 102° on the 33rd and 37th evenings, and to 103° on the morning and evening of the 38th day. No. 8433 recorded 102° on the evening of the 9th and morning of the 10th days, 103° on the 15th and 27th evenings, and again 102° on the evening of the 31st day. No. 8479 recorded 102° on the 5th and 9th evenings, 102.2° and 102.4° on the 21st and 22nd evenings, 104° and 103° on the 38th and 41st evenings, and 102.4° and 102° on the evening of the 43rd and morning of the 44th days respectively.

No well-marked clinical symptoms of the disease were apparent in these animals, but their blood when taken on the 49th day, mixed as above stated and injected in the quantity of 60 c.c. intrajugularly into horse 8294, caused the appearance of the disease in this animal after an incubation period of twelve days. The symptoms of the disease as shown by this animal were characteristic, the swollen or oedematous condition of the hind legs appearing about the 17th day, and the characteristic swaying or "wobbling" gait being in evidence on the 24th day, the conjunctival membrane presenting ecchymosis on a background at first pale and later rather dirty in appearance. The disease assumed a rather subacute form in this animal, loss of condition being evidenced by the fact that between the 3rd and 53rd day following the inoculation a loss of weight from 256 to 218 kilos. was

noted, and this animal when in the advanced stage of the disease was killed on the 77th day following inoculation for collection of material for pathological examination.

Conclusions.—The conclusion to be drawn from the above experiments is that, whilst we did not succeed in transmitting the disease to cattle, sheep, goats, or dogs under the conditions above stated, we proved its transmissibility to the mule and donkey in the experiments referred to immediately above.

Another experiment of this kind might here be mentioned, although, unfortunately, results obtained in this case were indefinite, and the experiment (prompted by the results of Japanese investigators) was not repeated. This experiment was made in injecting two pigs, Nos. 215 and 223, each with 10 c.c. subcutaneously of blood taken from horse 6435 on the 11th July, 1914. No reaction occurred in either of these animals. Their blood, taken on the 41st day, was mixed and injected in the quantity of 60 c.c. intrajugularly into horse 8230, but did not produce the characteristic symptoms of the disease in this horse, which died in a debilitated and anaemic condition on the 68th day. Its death as a result of pernicious anaemia was, however, queried in the absence of typical symptoms of the disease during life.

FILTRATION EXPERIMENTS.

Experiments were also made in attempting to transmit the disease to horses with the filtered blood or serum of affected animals, and these experiments are referred to as follows:—

1. Horses 7953 and 8467 were injected on the 15th January, 1914, each intrajugularly and with 200 c.c. of the serum of horse 7488 filtered through a Berkefeld filter, after dilution with physiological salt-solution (0.85 per cent.), the dilution being in the proportion of one part blood to ten parts salt-solution.

At the same time horses 3249 and 8284 were inoculated with a similar quantity of the diluted but unfiltered serum.

Result.

Horse 7953 showed a reaction which commenced on the 13th day, evening records of 104.8°, 104.8°, 105°, 104°, 104.4° being observed on the 13th, 14th, 15th, 16th, and 17th days, and from the 18th to the 22nd days the temperature could only be recorded on the 21st day owing to relaxation of the anus. Death occurred on the 26th day, but Nuttalliosis entered into this case as a complicating factor, *N. equi* appearing in the blood on the 18th day, being fairly frequent on the 20th day, and still present, though rare, on the 26th day, and therefore from this case no definite conclusion could be drawn.

Horse 8467 showed a temperature reaction following the injection from the 9th to the 16th day, and later reactions were also apparent, the animal ultimately being killed on the 84th day. The reactions observed in this animal were only slight and not of a very definite character.

Of the two control animals, horse 3249 accidentally contracted glanders and had to be killed on this account on the 24th day following the injection; horse 8284, however, showed a definite and characteristic reaction to the inoculation, the incubation period of the disease in this case being 12 days. The first temperature reaction lasted from the

12th to the 21st day in this animal, and following a remission between the 21st and 24th days another well-marked reaction set in and lasted up to the time when the animal was killed on the 37th day following the injection.

B. Horse 8574 was injected intrajugularly on the 21/2/14 with 500 c.c. of the filtrate obtained by filtering through a Berkefeld filter the defibrinated blood of horse 6594, after the dilution of this blood with physiological salt-solution (0.85 per cent.), the proportion of the former to the latter being 1 : 5.

At the same time horse 8531 was injected with 100 c.c. intrajugularly of the same diluted blood, which, however, in this case was not filtered.

Result.

Horse 8574 showed a definite and characteristic reaction following this injection, the first thermal reaction commencing on the 9th and terminating on the 14th days, with evening records of 104°, 103.4°, and 103° on the 10th, 11th, and 12th days. Another and prolonged reaction was subsequently noticed, and the animal—gradually but steadily losing condition, as evidenced by the difference in weights of 427 kilos on the 21/2/14 and 330 kilos on 6/5/14—was eventually killed when in a debilitated condition on the 22/5/14.

Horse 8531 also showed the symptoms characteristic of the disease, a first and slight reaction observed on the 9th, 10th, and 11th days, with evening records of 104°, 104°, and 103° respectively, being succeeded by a reaction from the 25th to the 34th days, the temperature during this time only descending below 103° on the 27th and 29th mornings, and reaching 104.8° on the 31st evening. From the 38th to the 44th days another well-marked reaction was apparent, and the animal eventually died from pernicious anaemia on the 66th day following the injection.

C. In horse 8564 the disease was produced by the subcutaneous inoculation on the 4/7/14 of 500 c.c. of the serum of horse 6435, after its dilution with physiological salt-solution, in the proportion of one part serum to nine parts salt-solution, and its subsequent filtration through a Berkefeld filter.

Following this injection, and from the 16th to the 20th days, a definite reaction occurred with higher temperature records of 103°, 105°, 103.8°, and 103.2° on the 17th, 18th, 19th, and 20th days, and subsequent and repeated reactions were also observed in this animal, which, however, did not die from the disease.

Conclusion.—The virus of this disease, present in the blood or serum of affected animals, and when these fluids are suitably diluted with physiological salt-solution, is capable of passing through the pores of a Berkefeld filter.

EXPERIMENTS MADE TO TRANSMIT THE DISEASE BY ADMINISTRATION THROUGH THE MOUTH, OF BLOOD OF AFFECTED ANIMALS.

Two horses were used for this experiment, viz., Nos. 5855 and 8504.

Horse 5855 received through the mouth, on the 20th February, 1914, 500 c.c. of the defibrinated blood of horse 6594 taken on the same date.

Horse 5855 showed typical and well-marked symptoms following the administration of this blood, the disease appearing with an incubation period of 16 days. The first thermal reaction lasted from the 16th to the 21st days, with maximum temperatures of 104° , 105° , 103.4° , and 103° on the 18th, 19th, 20th, and 21st days. A remission to the 29th day followed this reaction, but a thermal reaction setting in on the 29th day may be said to have lasted up to the time of the animal's death from pernicious anaemia on the 44th day, although slight remissions on the 35th and 36th days and again on the 41st day were noticeable.

This horse lost 40 kilos weight between the 4th and 33rd days following the administration of the blood, its weight on the 4th day being 396 kilos, on the 18th day 384 kilos, and on the 33rd day 356 kilos.

The other horse, No. 8504, showed, however, considerable resistance to this method of infection, and though eventually showing a slight reaction following the last administration of blood by the mouth, later appeared to recover, and was still alive on the 5th January, 1915.

This horse received as a drench 500 c.c. of defibrinated blood of horse 7625 on 10/6/14, but showing no reaction again received in the same way 500 c.c. of defibrinated blood of horse 6435 on 3/7/15. No reaction to this latter administration being apparent, it again received 900 c.c. of a mixture, in equal parts, of the defibrinated blood of horses 8443 and 8564 on the 24th July, 1914. Following this a slight reaction was noted on the 14th and 15th days, again from the 28th to 31st days, and later, on the 44th and 45th and 46th days. A slight irregular reaction was again noted between the 66th and 71st days. A slight irregular reaction was again noted between the 66th and 71st days, but following this no definite reactions were observed up to 5th January, 1914.

Conclusion.

This disease can be experimentally transmitted to the horse by the administration through the mouth of the blood of an animal suffering from the disease.

EXPERIMENTS MADE TO TRANSMIT THE DISEASE BY MEANS OF THE URINE OF AFFECTED ANIMALS.

Two horses were used in this experiment, viz., Nos. 5856 and 8538.

Horse 5856 did not contract the disease, although injected subcutaneously with the urine of horse 6594 on the 20th February, 1914, in the dose of 500 c.c., and again with 500 c.c. subcutaneously on the 4/7/14 with the urine of horse 6435. Local reactions in the form of large swellings at the site of inoculation followed both of these injections, but no other reaction was observed, save a slight rise of temperature during the time the local swellings were present, and the animal was eventually killed on the 22/8/14.

Horse 8538, however, received the urine of affected animals by oral administration on a number of occasions, and, finally contracting the disease, died as a result on the 36th day following the last administration of urine in this manner.

Thus, on the 10/6/14, this horse received 300 c.c. by the mouth of the urine of horse 7625. No reaction being apparent, however, up to the 4/7/14, it again received, in the same manner, 500 c.c. of the urine of horse 6435. No reaction appearing up to the 17/7/14, it again received by the mouth 600 c.c. of urine of horse 8443 on this latter date, and on the following day (18/7/14) 600 c.c. of the urine of horse 6435 was administered by the same route.

Five days afterwards (23/7/14) and in the absence of any reaction, it again received by the mouth 500 c.c. of a mixture of urine of horses 8443 and 8564, in the proportion of 1:3 respectively. Following this last administration and with an incubation period of 25 days, a definite reaction with typical symptoms of the disease appeared, the animal finally dying of pernicious anaemia on the 36th day following the last administration of urine by the mouth.

Conclusion.

This disease can be experimentally transmitted to the horse by the administration through the mouth of the urine of animals affected with the disease.

GENERAL CONCLUSIONS AND SUMMARY.

The general conclusions that therefore appear to be warranted from the results of the foregoing experiments are, that this disease appearing in horses is capable of being transmitted to other horses by the inoculation of the blood or serum of affected animals; that the virus present in the blood or serum of affected animals is capable under suitable conditions of passing through the pores of a Berkefeld filter; that the disease can be transmitted to the mule and the donkey; that we were unable to transmit it to cattle, sheep, goats, or dogs under the conditions of our experiments; and that it could be experimentally transmitted to horses by the administration through the mouth of blood or urine of affected animals when relatively large quantities of these fluids were used.

The period of incubation of this disease following inoculation may be as short as five days, but, varying between this and twenty-five days, incubation periods of from ten to fifteen days are not uncommon, and in one instance the length of this period appeared to be thirty days. Further, the length of this period does not seem to be in any direct relationship to the type of disease which follows.

A point of marked interest, however, to which we wish to draw attention is the relationship existing between this disease and horse-sickness, since it has been suggested by certain European workers that infectious or pernicious anaemia possibly represents a modified or mild form of horse-sickness, a suggestion which appears to have been based on an observation that a horse recovering from pernicious anaemia later proved refractory to infection by a particular strain of horse-sickness virus with which it was injected. The evidence on the point, however, was regarded as inconclusive, and, to our knowledge, no definite evidence for or against this view has since then been brought forward.

Our experiments bear directly on this point, and furnish evidence to show that this view above referred to is incorrect, since the majority of horses in which the disease was produced in these experiments were horses not only immune to different virulent strains of horse-sickness virus, but animals which had even been hyperimmunized to the

extent of 10 litres given intrajugularly with the virulent blood of horses passing through horse-sickness reactions induced by the injection of markedly virulent strains of horse-sickness virus.

We, therefore, from our experiments cannot support the view that infectious or pernicious anaemia represents a modified mild form of horse-sickness, but are forced to regard the two diseases as entities separate and distinct in themselves.

Another point which the consideration of our experimental observations bring us to remark upon, and which may be noted here, is the apparent unsuitability of the terms infectious or pernicious "anaemia" as descriptive appellations of the disease, for although the disease is definitely infectious, and anaemia is apparent in the chronic form, still anaemia is not necessarily a well-marked clinical symptom in animals suffering from the more acute form of the disease or in those animals which, though clinically recovered, may yet remain virus "carriers" or "reservoirs."

This latter observation in regard to the absence of anaemia in acute cases is one to which reference has also been made by other investigators, but we have here retained the names of infectious or pernicious anaemia to denote the disease as being names by which it is already widely known, and under which it is most commonly referred to in the literature dealing with the subject.

We shall now proceed to review the general symptomatology of the condition, and in doing this we propose to separately discuss the symptoms as they occur in its acute, sub-acute, and chronic forms.

SYMPTOMATOLOGY.

Acute form.—Under this form we describe those cases observed in which an acute attack of the disease terminated in the death of the animal after a course varying from about thirteen to seventeen days. In a couple of cases, however, the course observed was of longer duration, and death did not supervene until about the twenty-fourth day from the onset of symptoms.

It should also be noted that a primary acute attack need not necessarily terminate in death, but may be succeeded by either the sub-acute or chronic form of the malady.

The first symptom noted in acute cases is the rise of temperature, occurring after a period of incubation varying as before stated. This primary rise in temperature may be noted in connection with either the morning or evening record, and, from the time of its appearance onwards, the temperature remains more or less elevated up to the time of death, often remaining between 104° F. and 105° F. for days, with maximum records lying generally between 106° F. and 107° F. The curve described is, however, not very regular in outline, since at times depressions occur marking the occurrence of remissions lasting from about twenty-four to forty-eight hours, and these remissions may cause a difference of from three to four degrees or more between the evening of one day and the morning of the next. In some cases there was also observed the appearance of a first and lesser reaction lasting generally for a few days, which was then succeeded by a remission of about a couple of days' duration, and then following this there occurred the development of a second and more marked reaction which ultimately terminated in death. Towards death the curve may either remain comparatively high, or show a

tendency towards a fairly gradual descent, and it is not usual to find a sudden fall through several degrees to sub-normal as it is met with, for instance, in the "dikkop" form of horse-sickness. In some cases, however, the rectal temperature could not be recorded owing to relaxation of the anal sphincter, and this, though more usually occurring towards the time of death, was also noted to occur in some cases at varying times during the reaction.

Outside of the temperature reaction other symptoms are also to be noted, and these are usually met with from about the day following the initial temperature rise onwards, and may be described as follows:

The animal appears more dull than usual and the appetite is decreased or may be absent. In the stable it may stand back from the manger, with the head drooping slightly, and resting the weight of the hindquarters alternately on either hind limb, the limb on the side not supporting weight being flexed, with the toe resting on the ground, and the quarter on that side being lower than the other.

The pulse may be increased in frequency, and be as frequent as 70, 80, or even 100 per minute, but very frequently it only varies very slightly around 50, and it may be remarked that the frequency is not necessarily increased in proportion to the elevation of temperature, since with this latter in the neighbourhood of 105° F. or 106° F., a pulse rate varying between 45 and 50 per minute is of frequent occurrence.

The conjunctiva in the earlier portion of the attack may present a rather orange coloration, the vessels being at the same time injected, and petechiae are usually present in addition. Later, however, the orange coloration may give way to a dirty or muddy appearance, or a rather washed-out red colour, and the membrane then may at the same time appear rather oedematous.

The mucous membranes of the mouth may also be injected, or sometimes may present a dirty yellowish colour, but if, as is sometimes noted, both these appearances tend to occur at the one time, the coloration then resulting is one which is reminiscent of that met with in certain cases of equine influenza, where it is sometimes referred to as "brick-red."

In some cases, however, the mucous membranes of the mouth may present a paler yellowish colour.

The respirations are sometimes increased and may be as frequent as 20 or 30 per minute or slightly more, but no specific changes in the lungs can be determined either by auscultation or percussion.

Loss of condition may be, and usually is, fairly rapid in onset and progress, and may be apparent after the first few days of the febrile period, being especially noticeable in the musculature of the hindquarters and continuing to occur up to the time of death. Very marked loss, however, is not a feature of this form of the disease and actual "wasting" does not occur, many animals coming to autopsy in comparatively good condition, and though a slight decrease in the blood-cell count and hæmoglobin content of the blood may be recorded, yet death can occur without either of these values being markedly altered, both of these facts serving to show that anaemia is not a clinical feature of the acute form of the disease.

A loss of muscular power also becomes apparent within a few days from the time of onset of other symptoms, and this condition may become very marked within a day or two after its first appearance,

being especially noticeable in connection with the hindquarters and limbs.

This loss in power or paresis of the muscles of the hindquarters causes the animal to walk with a very uncertain gait and swaying movement of the posterior part of the body, the hind feet being trailed along the ground. There is great difficulty in turning, and if the animal is forced to turn quickly the hindquarters sway and the animal appears as if about to topple over or fall. Sometimes there is noted, in addition, an uncertain stiff gait in the fore legs and a sudden flexion of the fetlock-joints ("knuckling-over") may be also present, and in these cases movement then becomes extremely difficult, the gait assumed being of a rather staggering character.

When the animal presents these symptoms and is forced to move, it is only necessary to walk it a very short distance to cause a fairly marked increase in the pulse rate, the heart-beat at the same time usually becoming intermittent and more forcible than usual or perhaps even palpitating in character, and in some cases a jugular pulsation has also been noted to occur.

Oedema of dependent parts of the body cannot be said to be a marked symptom in this form of the disease, but as the disease progresses a certain amount of oedema may appear, which is manifested by a swelling of the hind legs or perhaps even of all four legs, and in some cases a slight oedematous swelling underneath the chest or abdomen may at the same time be also seen.

In addition, a "tucked-up" condition of the abdomen may be present, and a decrease or loss in response to digital pressure in the lumbar region is also to be noted.

The urine is generally very dark yellow in colour, but neither hæmoglobinuria nor hæmaturia is present in uncomplicated cases. Examination for albumin reveals its presence, but not in larger quantities than might be expected in conditions showing similar febrile symptoms, and on this point we find ourselves in concordance with American observers in never having met with the quantities mentioned by certain European investigators, nor were quantities as large as those noted by the Japanese observers ever noted to occur.

A slight looseness of the bowels and even slight diarrhoea was noted in one or two cases, but this as a symptom was never very marked and in the cases where met with was of very short duration.

Towards the end the animal goes down, becomes unable to rise, and relaxation of the sphincters may occur. In the majority of cases, however, the rectal temperature could be recorded up to shortly before the time when death occurred.

Sub-acute Form.—This form of the disease is characterized by the occurrence of a repeated number of attacks somewhat similar, though perhaps not always so severe as those described in the acute form, with, in addition, the appearance of intermittent remissions lasting over periods varying in length from several days to may be weeks.

The symptoms of these exacerbations resemble fairly closely those described in the acute attack, and hence there is no need to mention them in detail, but here is given a general description of the other symptoms met with which go to form the clinical picture of this type of the disease and thus assist in its differentiation from the other two forms encountered. Thus it may be noted that the mucous membranes

are generally somewhat paler than in the acute form, and the conjunctiva has more usually the washed-out appearance already referred to, with perhaps petechiae present in addition, though during the febrile attacks the membranes may appear to be somewhat injected and the appearances just noted be then not so apparent as during the remissions. In one case we have noted the appearance of a blood-tinged serous discharge from the conjunctival sac appearing at the inner canthus and associated with ecchymosis of the conjunctiva.

Similarly to what is observed in the acute form, with the increased temperature of the febrile attack, there is usually associated loss of appetite and condition, and increased frequency of the pulse and respirations and slight albuminuria are symptoms which may be also noted to occur.

Oedematous swellings of the legs and under the chest and abdomen and of the prepuce are conditions also to be met with, as well as an uncertain gait due to muscular paresis as noted in the acute form of the disease.

During the remissions the animal may improve, the appetite be regained, and recovery appear as if about to occur, but the muscular weakness, the somewhat pallid or washed-out appearance of the mucous membranes, and the oedema of dependent portions of the body may persist until a subsequent attack occurs.

This form of the disease may in this way last for many weeks or even months, and may finally terminate in death during one of the exacerbations. On the other hand, however, it may pass into the chronic form, or another possibility which is existent is that the attacks may grow less and less severe and more widely separated and even finally perhaps cease altogether to appear, the animal in the meantime improving markedly in condition ultimately coming to appear as if clinically recovered from the disease.

Chronic Form.—In this form the occurrence of temperature elevations with intercurrent remissions is also to be met with, but, unlike the sub-acute form, these reactions are not associated with other marked symptoms special to these periods, and a further difference is that in this form the presence of anaemia is a well-marked feature.

The chronic form may appear as a sequel to a primary acute attack, or to the sub-acute form, or the disease may take on this type more or less from the commencement, none of the reactions observed being of a very marked character. The interfebrile periods may vary from weeks to months, and during the febrile attacks the temperature usually rises rapidly, may be running up to 105° F. or 106.5° F. in about twenty-four hours, and then, after remaining high for a couple of days or so, may again drop back to normal inside 24 to 36 hours.

During these attacks, however, the animal shows little of the other symptoms that may in other cases be associated with elevated temperature, and though the pulse may be increased in frequency to about 70 per minute and be intermittent, and the cardiac sounds be irregular, yet, on the other hand, the pulse may not be especially disturbed.

These reactions may be noted to recur over very long periods, and amongst our cases we have noted that they occurred even up to eight months after the animal first became affected.

During these attacks weight and condition is generally decreased, and though in some cases a certain amount may be regained during

the subsequent remission and thus the condition may remain fairly constant, yet in the majority of cases, either the weight regained during the remission falls short of that lost during the temperature reaction or a loss of condition during the remission is also noted.

In both these cases therefore, gradual wasting and emaciation occur, and thus it is that anaemia and emaciation come to be characteristics of this form of the disease.

The affected animals are in poor or may be very poor condition, with a hide-bound and often staring coat, and are languid and not inclined to move rapidly. The gait is "wobbling" and uncertain owing to muscular weakness, and if the animal is forced to trot for a very short distance the pulse frequency is increased to an excessive degree, the heart's action becoming palpitating and visible from a little distance, and, at the same time, the cardiac sounds may become irregular and a jugular pulsation be often seen.

The mucous membranes are very pale, though a slight yellow tinge is sometimes apparent during the period of temperature elevation, and for a short time afterwards in some cases, and petechiae are, as a rule, present on the conjunctiva.

The blood-count is often reduced in this form, and red-cell counts of two millions per c.mm. have been noted. The hæmoglobin value and viscosity of the blood are also reduced, and a very slight anisocytosis may also be met with, but polychromatic cells are extremely rare; basophilia has never been met with, nor have normoblasts or megaloblasts ever been seen.

The appetite is, as a rule, not interfered with and may be very well marked.

Oedema of dependent parts, as evidenced by swelling of the hind legs, is usually present, and in some cases swellings of the prepuce and underneath the abdomen are also met with.

Examination of the urine for albumin during the interfebrile periods proved negative, and during the febrile periods it was only met with in some instances, and then only in traces.

The termination of this form of the disease is that death occurs from debility and exhaustion following upon the anaemic condition present and during a remission, or, as we noted in a few instances, at the end of a febrile attack of more marked type than usual.

THE BLOOD OF AN ANIMAL APPARENTLY CLINICALLY RECOVERED MAY STILL REMAIN INFECTIVE FOR A LONG PERIOD.

This concludes a general description of the symptomatology of the disease, but, in speaking of the sub-acute form, mention has been made of the fact that though an animal may clinically appear to recover, yet its blood may still remain infective, and to illustrate this point particularly special reference may be made here to horse 6576.

This horse had been first injected on 10/10/13 with virus Ziervogel 7483, and, following this, no reaction having been observed, it was again injected on the 27th October, 1913, with virus Onderstepoort 7781. No reaction followed this latter injection up to the 21/11/13, and on this latter date the animal was then injected for a third time, this time receiving the virus McCall (59) 7348.

From this latter injection this animal contracted the disease, and a very marked reaction occurred between the 8th and 27th days following the injection, the temperature for the greater part of the reaction remaining in the neighbourhood of 104°, but reaching 105°

on the 16th and 17th days, 106° on the 18th day, 105.2° on the 20th day, and 104.8° on the 25th day, and showing slight remissions on the 11th, and again on the 22nd and 23rd days.

The temperature then descended to a lower level, and as a rule did not exceed 102° up to the 64th day. From the 64th to the 68th days, however, another reaction occurred, with maximum records of 104.8° and 105° on the 65th and 66th days.

Three further reactions were also noted. One of those occurred between the 82nd and 88th days, with higher records of 105° , 105° , 105° , 104.6° , and 104° on the 82nd, 83rd, 84th, 86th, and 87th days; another occurred between the 98th and 103rd days, with higher records of 103.4° , 104° , 104.8° , and 104.6° on the 98th, 99th, 101st, and 102nd days; and the third reaction was noted between the 134th and 141st days, with higher records of 104.6° , 107° , 106° , 105° , and 103.4° on the 135th, 136th, 137th, 138th, and 139th days.

Following this last-mentioned reaction no further reactions were noted in this animal, and, though it had lost a certain amount of condition during these attacks, it now steadily improved, and finally appeared to have recovered completely, its condition ultimately being very good.

That its blood, however, still remained infective in spite of this apparent recovery was shown by the fact that on removing 20 c.c. of its blood on the 221st day following the inoculation producing the disease and 80th day from the termination of the last febrile reaction, this quantity of blood produced the disease in horse 7785 on intrajugular injection, the disease in this latter animal assuming a sub-acute form, and terminating ultimately in its death from infectious anaemia.

Similarly also in the case of another animal which appeared in very good condition (horse 6435), we were able to prove that its blood was infective on the 229th day from the time of the inoculation which produced the disease, and this was shown by the inoculation of horse 8443 intrajugularly with 20 c.c. of the blood of horse 6435 taken at the above time, for, as a result of this injection, horse 8443 developed the acute form of the disease after an incubation period of fourteen days, and finally died from infectious anaemia on the 33rd day following the injection.

In the case of horse 6435, however, though appearing outwardly normal and in good condition, its behaviour in regard to temperature was different to that of horse 6576, for reactions continued to occur for a very long period following the inoculation producing the disease, one of these being noted immediately subsequent to the time of withdrawal of blood for the above inoculation, and from the 230th to the 234th days, and even beyond this time reactions were again noted to occur, as, for instance, between the 289th and 291st days, later between the 436th and 439th days, still later between the 477th and 481st days.

ATTEMPTS AT CURATIVE TREATMENT.

During the progress of certain of the cases attempts at curative treatment were made in certain instances, but the drugs used did not appear to exercise any appreciable beneficial effect on the course of the disease.

Some of the medicinal agents whose action was tested should, however, be referred to, and this may be done as follows:—

In some cases formalin was used. Thus horse 5782 received on one occasion 1 c.c., and 16 days later 5 c.c. by intrajugular injection, the formalin being mixed with one litre of physiological saline solution. Similarly administered, horse 7412 received 2 c.c. during the course of the disease; horse 6594 received 2 c.c., followed 15 days later by 5 c.c.; horse 6529 received 3 c.c., and 10 days later 5 c.c.; horse 6435 received 4 c.c.; and horse 6432 received 5 c.c. In other cases iodine in solution with potassium iodide was used. Thus horse 7592 received 1 gramme of the former, plus 5 grammes of the latter; horse 7417, 2 grammes plus 5 grammes; horse 7293, 2 grammes plus 5 grammes; and horse 7077, 4 grammes plus 5 grammes, the solutions in all cases being made in one litre of physiological saline solution and given intrajugularly. Combinations of these treatments were also tried. Thus horse 7145 received 2 c.c. formalin, and 6 days later 1.3 gramme iodine with 5 grammes potassium iodide; horse 7076 received 2 grammes iodine with 5 grammes potassium iodide, and 13 days later, 5 c.c. formalin; and horse 6766 received 0.5 gramme iodine with 2.5 grammes potassium iodide, and 18 days later, 5 c.c. formalin. The iodine and potassium iodide was in each instance dissolved in 1 litre of physiological saline solution and given intrajugularly, the formalin being administered in a similar fashion. Arsenophenylglycin was also used, two horses, Nos. 7331 (385 kg.) and 6541 (290 kg.) respectively receiving 23 grammes and 17 grammes, given intrajugularly in 500 c.c. of physiological saline solution, and in one instance novoflavin, horse 7442 (351 kg.) receiving intrajugularly 3.5 grammes in 500 c.c. physiological saline solution. In another case, that of horse 6470, the animal first received 5 grammes quinine hydrochloride with 5 grammes methylene blue, then seven days later 3 c.c. formalin, and four days after this, 3 grammes iodine with 5 grammes potassium iodide, the agents used on each occasion being given intrajugularly in solution in one litre of physiological saline solution.

Another horse, No. 7156, received intrajugularly 10 grammes of quinine hydrochloride in 1 litre physiological saline solution, but died from quinine poisoning as a result.

Horse 7346 (285 kg.) received intrajugularly 10 grammes of methylene blue in 1 litre of physiological saline solution. Two days later this animal died and hæmoglobinuria was noted on post-mortem, smears from the spleen, however, failing to reveal parasites.

Horse 7066 received 3 grammes of iodine and 5 grammes potassium iodide intrajugularly in 1 litre of physiological saline solution. Five days later *N. equi* appeared in the blood, and it was then injected intrajugularly with 200 c.c. of a 1 per cent. solution of trypan blue, but death occurred on the same day.

This serving to indicate the nature of attempted treatment, we now pass to a description of the anatomo-pathological appearances encountered at post-mortem examination of animals dying from the disease.

POST-MORTEM APPEARANCES OF ANIMALS DYING FROM THE DISEASE.

In describing the post-mortem appearances it must be pointed out that no specifically characteristic changes have ever been encountered in any of the animals coming to autopsy, but in the following

notes there will be found a description of the general anatomo-pathological picture which may be expected to be present in animals dying from the various forms of the disease.

In animals dying from the *acute* form, the condition of the cadaver varies, depending on the condition of the animal immediately previous to contracting the disease and may be quite good, and the development of fat in the subcutaneous tissues and elsewhere varies in a similar fashion.

Abrasions of the skin may be present if the animal has been down and unable to rise towards the time of death. The natural openings present nothing unusual.

The visible mucous membranes may have a slight yellowish or dirty yellowish tinge, and the blood may have a slight brownish tinge and be imperfectly coagulated. A varying degree of icterus, indicated by a brownish-red coloration of the flesh and the fat and connective tissue being yellower than normal, is also to be met with.

In the peritoneal or pleural cavities, or both, a varying, though as a rule small, quantity of liquid may be present, and this is often of a deep yellow colour, a reddish tinge being sometimes noted, but usually only when the autopsy has been delayed.

The pericardial sac may be empty, but often contains 10-30 c.c., and maybe up to 200 c.c., of a liquid similar to that present in the other serous cavities.

The heart generally presents hæmorrhages of varying size underneath the epicardium, and the same condition may be met with in both ventricles in the form of sub-endocardial hæmorrhages, these being occasionally also met with in the auriculo-ventricular valves. The blood contained in the heart is generally imperfectly coagulated, and the myocardium is usually of a paler or more greyish brown colour than normal, and being also more opaque and softer in consistency than usual, thus presents the parboiled appearance indicative of "cloudy-swelling" or parenchymatous degeneration.

No special lesions have been noted in connection with the blood-vessels.

Under the parietal and visceral layers of the pleurae, petechiae ecchymoses, and extravasations of varying size may occur, and hæmorrhages in the mediastinum have also been noted. The membranes are otherwise smooth and glistening.

The lungs may be incompletely collapsed, and show a certain amount of hyperaemia on section, and a slight pulmonary oedema has also been seen.

The mucous membrane of the larynx, trachea, and bronchi may be rather pale or palish yellow in colour, but injection of the vessels may be present and even small hæmorrhages be seen, and where pulmonary oedema is present the bronchi may also show the presence of a small quantity of foam.

The mediastinal, bronchial, and prepectoral glands show a varying amount of enlargement, oedema, and hyperaemia, and a hæmorrhagic appearance may even be presented.

In the peritoneal cavity, sub-peritoneal petechiae, ecchymoses, and even larger hæmorrhages may be met with in different situations, but these have been most frequently seen under the visceral layer of the peritoneum and then over the large colon and coecum.

The liver is usually somewhat enlarged, and on section of brownish colour with sometimes a slight yellowish tinge, and in

addition is friable in consistency, more opaque than usual and the lobulation is generally fairly well marked. The portal lymph gland is, as a rule, swollen and hyperaemic, and may on section even present the appearance of hæmorrhages.

In all cases the bile duct and its duodenal orifice were patent.

The pancreas presents nothing unusual.

The spleen is enlarged, and frequently subcapsular hæmorrhages are present. On section the pulp is swollen and dark red or blackish in colour, and is generally soft or moist, the trabeculae not being distinctly visible. It may, however, be swollen and yet of fairly firm consistency. The splenic lymph glands also are enlarged and oedematous, and hyperaemic or even hæmorrhagic on section.

In the stomach, the mucosa of the pyloric portion may present no unusual appearance, but there may be present hyperaemic patches and, in some cases, even hæmorrhages into the mucous membrane are to be seen.

The small intestines may show thickening of the mucosa, which latter is of a somewhat yellowish colour, and hyperaemia in patches, stripes, or streaks is usually present. In addition to this hæmorrhages of various size are often seen in the mucosa, and in one case a blood tinging of the liquid bowel contents was noted.

In the large intestines a similar appearance is to be encountered, hyperaemic areas and hæmorrhages, as above described, being there also to be met with.

The pharyngeal and mesenteric lymphatic glands present appearances similar to those of the thoracic and other glands, and are usually enlarged and oedematous, and on section present a hyperaemic or even hæmorrhagic appearance.

The tongue presents nothing very unusual, though the musculature may perhaps have a more brownish tinge than normal, and similarly nothing very unusual is noted in connection with the salivary glands, pharynx, or oesophagus, or with the thyroid or suprarenal bodies.

In the urogenital system the kidneys are generally enlarged, softer than usual, and the capsules can be easily detached, and on the surface of the cortex small hæmorrhages may be apparent. On section they may present injection of the cortical vessels, and sometimes even small hæmorrhages into the cortex, and generally they are somewhat more opaque and slightly paler than normal, these latter appearances indicating the presence of parenchymatous degeneration.

The urine contained in the bladder is generally of a dark yellow colour, and the vesical mucous membrane usually shows injection of the vessels and hyperaemic areas, and in some cases actual hæmorrhages.

The brain and eyes present nothing markedly unusual, and hæmorrhages into or under the retina have not been seen.

In the *sub-acute* form of the disease, the appearances met with vary fairly considerably, depending on the course the disease assumed during life, and whilst in some cases the appearances met with approximate towards those seen in the acute form, in others the changes met with approach more towards those seen in the chronic type.

In cases of the first type are included those which showed a fairly good condition of the cadaver; slight icterus; hæmorrhages under the serous membranes, as well as hæmorrhages into the mucous

membranes of the gastrointestinal tract and bladder and into the parenchyma of certain organs, such as kidneys and lungs; well marked enlargement of the spleen, with swelling and softening of the pulp; an enlarged, oedematous, and hyperaemic or even hæmorrhagic appearance of the lymphatic glands; and parenchymatous degeneration of the heart, kidneys, and liver, this latter also often presenting the yellowish-brown colour noted in acute cases.

The other type of cases comprises those in which was found a poorer condition of the carcass; a certain degree of paleness of the mucous membranes and of the flesh; hæmorrhages, as above described, either absent or extremely rare; a not very well marked enlargement of the spleen; and the lymphatic glands enlarged, oedematous, and juicy on section, but not showing very marked hyperaemia. Lesions of parenchymatous degeneration in heart, liver, and kidneys are also to be met with.

In animals which die from the *chronic form*, the carcass is in poor condition and usually emaciated, with lesions of decubitus over the bony prominences usually present, and, as a rule, oedematous, or more frequently gelatinous, infiltrations of the connective tissues, especially of the more dependent parts, are also to be seen.

The natural openings, as a rule, present nothing unusual, save sometimes a relaxation of the anal and vulvar sphincters, and the visible mucous membranes are very pale.

The fat in the subcutaneous and other connective tissues is very poorly developed or generally almost absent. The blood is thin and rather watery and does not stain well, and plasma coagula are often present in the heart cavities. The musculature is pale, though a certain amount of brownish pigmentation is also present.

A varying, though, as a rule, small amount of clear liquid may be present in the peritoneal cavity, and the same may also sometimes be met with in small quantity in the pericardial sac.

The heart may show parenchymatous degeneration or as is more usually the case slight fatty degeneration, a varying amount of atrophy and brownish pigmentation of the myocardium having been also noted to occur, and the fat at the base of the heart and along the coronary furrows is replaced by a mucoid or gelatinous tissue.

The lungs are generally collapsed and usually present nothing unusual, though a certain amount of stasis may be present. In a number of cases, however, thrombosis of the pulmonary arteries was met with, and this was often associated with a varying degree of thrombosis in the iliac and femoral arteries.

The liver may be of normal size or somewhat contracted or atrophied, appearing of firm consistency outwardly, and often somewhat bluish in colour. On section the colour is rather paler and the parenchyma more opaque than usual, and generally a slight fatty degeneration and a varying degree of brownish pigmentation of the periphery of the lobules is present. The lobulation is well marked, Glisson's capsule being always distinct and sometimes even appearing to be slightly increased.

The spleen may be of normal size or appear slightly contracted, but is never enlarged. On section the pulp is dry, firm, and of a brownish-red or brownish colour, and the trabeculae are distinct, and in some cases a certain degree of enlargement of the malpighian bodies has been noted, these then being very distinctly visible and greyish in colour.

The kidneys are often of usual size, but may be slightly smaller than usual, and the capsules are freely removable. On section they are paler than usual, somewhat bloodless, and show appearances of albuminous or more frequently a slight fatty degeneration. The vesical mucous membrane is pale.

The lymphatic glands of the body may be more or less normal in size, but are sometimes slightly enlarged through oedema, and on section are then somewhat juicy, being in most cases pale. Sometimes however they are slightly enlarged and firm and on section present a rather marbled appearance with areas of greyish-yellow and pale-reddish coloration, and this condition is usually best seen in the splenic lymph glands though the others may present a similar appearance.

The mucous membranes of the various portions of the alimentary tract are in general pale, but in the case of animals which have been affected with decubitus and accompanying intestinal stasis some days before death, patches of hyperaemia of varying size may frequently be noted in the intestinal mucosa.

Other conditions met with at autopsy and common to all forms of the disease have not been specially referred to since they are also to be met with in the case of nearly all animals coming to post-mortem examination.

Some reference may, however, be made to them, and they may be mentioned as being met with in the presence of fibrous filamentous outgrowths from the anterior surface of the liver capsule, from the serosa of the intestines (especially that of the colon and coecum), the surface of the diaphragm, and from the pulmonary pleurae; in the presence of patches of fibrous thickenings of the liver capsule and pulmonary pleurae; in the occurrence of gastrophilus larvae in the stomach and sclerostome larvae in the anterior mesenteric artery in practically all cases, and in some cases of gastrophilus larvae in the duodenum; in the occurrence of sclerostomes in the coecum and colon and at times of tumours in the gastric lining due to spiroptera; also in the frequent presence in the peritoneal cavity of worms of filaria species; and in some cases the occurrence of calcareous nodules in lungs, liver, and may be the intestinal wall.

The appearance presented by the bone-marrow has not been spoken of above in describing the anatomo-pathological appearances of the disease, but is referred to separately in the following section of this present article, since for certain reasons it appears worthy of more detailed consideration than that given the other conditions just dealt with.

The Appearance of the Bone-Marrow.—In this section of the present article the appearance of the bone-marrow is particularly dealt with on account of the fact that some writers describe the bone-marrow in this disease as presenting changes or appearances which may, to a certain extent, be regarded as characteristic of the condition, and especially when other lesions present on autopsy are only those of a septicæmic nature or where, for other reasons, the existence of the disease is suspected.

Thus both French and German authors refer to the constant changes present in the marrow of the medullary cavity of the long bones of the upper extremities, and French writers speak of the bone-

marrow, especially of the femur, as presenting a brick-red or black discoloration, and a consistency and histological appearance resembling that of foetal marrow, whilst Hutyra and Merek speak of the marked and constant changes in the bone-marrow of the proximal long bones of the extremities especially in the femur and humerus, which they state, are indicated by a brownish-red, or blackish-red, colour of the bone-marrow, with either a part or the whole of the marrow involved in the change. The spongy-marrow of these bones is also said to present a reddish coloration, and the spongy-marrow of the vertebrae, ribs, etc., is said to present a similar appearance. In cases with a longer and slower progress, it is stated that the reddish areas in the fat-marrow are more circumscribed, and that in some cases the marrow may appear to be normal, but even in very chronic cases small reddish areas may still be met with, and these areas of reddish discoloration they, however, point out are not hæmorrhages, but are indicative as in other cases of anaemia of a regenerative process involving an increase in the activity of the red-marrow blood-forming cells, the normal occurrence of which, they state, is described by Skiba in the fatty or gelatinous marrow of the femur and humerus.

Ostertag and others also refer to a similar appearance of the bone-marrow, and the Japanese Commission state that "changes of the bone-marrow are very marked next to those of blood. In the acute cases the cross-section of long bones, such as the femur or humerus, shows that the bone-marrow changes wholly or in part into dark-red with petechiae. In the chronic cases the change is not so extensive as in the acute form, but it develops as the foci. In the protracted cases a small number of foci are invariably present."

Van Es, Harris, and Schalk, having made a very complete review of the findings of others as well as themselves, point out that there are no lesions which can be said to be pathognomonic of the disease, and state that "the features deserving most attention are the petechiae and ecchymosis of certain structures, lymphnode involvement, interstitial and parenchymatous lesions of certain important viscera, albuminuria, and the alteration of the bone-marrow and of the blood. . . . It is possible (they say) that the bone-marrow lesions constitute a valuable aid in the diagnosis of the disease, but, in view of the fact that we know comparatively so little of bone-marrow changes in connection with other diseases of the horse, or even under varying normal conditions, we would rather suspend judgment relating to their diagnostic value until the matter has been looked into more fully."

In view of all of the above evidence we therefore considered it advisable to examine the appearance of the bone-marrow, not only in cases of animals dying from infectious or pernicious anaemia, but also in the case of those which died from other causes or were killed. In a number of animals therefore the bone-marrow of the humerus and the femur was specially examined with this object in view, and it must be here stated that the results obtained do not allow us to regard the marrow of animals dying from pernicious anaemia as presenting any specially characteristic appearance, since exactly similar appearances are to be met with in animals coming to autopsy as a result of death from a large variety of other causes. Believing that it may be of interest to record some of our findings in this connection, we have here set forth a summary of results of the exami-

nation of the bone-marrow of 201 animals coming consecutively to post-mortem examination.

These cases we have grouped collectively according to the appearance of the marrow of the femur and humerus presented by the individual horses, the post-mortem diagnosis and condition of the cadaver of the different cases being also given, and in summarizing these cases the groups into which they fall are referred to under different headings, the significance of which may be here explained. Thus, in group 1, referred to under the heading "fat-marrow," have been placed those cases in which the marrow was purely of a fatty character, no red areas being noted. In group 2, "fat and red marrow," are included all those cases in which red marrow in varying quantities was present in the medullary cavity and also in the spongiosa, and in many of these cases it may be remarked that red-marrow formed $\frac{2}{3}$ to $\frac{3}{4}$ of the total marrow present in the former situation. It is to be remarked, however, that whilst the red coloration of the marrow of the spongiosa of the proximal epiphysis of the femur is of common occurrence, the same does not apply to the distal epiphysis. A similar statement applies generally in the case of the humerus, but here it should be also added that the amount of red-marrow in this latter bone is generally not present in a quantity proportionally so great as in the femur of the same individual. In the third group, that which is referred to under the heading of "gelatinous-marrow," have been placed cases where the marrow presented a uniform gelatinous or jelly-like appearance with a yellowish or rather greenish-yellow coloration, and, finally, we have placed under the heading of "gelatinous and red marrow," those cases which resembling those in group 2 in regard to the distribution of the varieties of marrow present differed from them in that here the fat-marrow was replaced by a gelatinous material similar to that forming the total marrow of the last group of animals.

The significance of these various headings having been explained, the results obtained from the consideration of the 201 cases examined may now be set forth in detail as follows:—

(1) *Fat-Marrow.*

No. OF CASES.	POST-MORTEM DIAGNOSIS.	CONDITION.			
		Good.	Fair.	Poor.	Very Poor.
4	Horse-sickness.....	1	3	—	—
1	Glanders.....	—	1	—	—
1	Sarcomatosis (liver and lungs).....	—	—	1	—
1	Colic.....	—	—	1	—
3	Killed.....	—	1	1	1
10		1	5	3	1

(2) *Fat and Red Marrow.*

No. of Cases.	Post-mortem Diagnosis.	Condition.			
		Good.	Fair.	Poor.	Very Poor.
69	Horse-sickness (including 1 sequel horse-sickness and 1 case complicated with hæmaturia).....	22	30	17	—
19	Pernicious anaemia.....	3	5	10	1
16	Killed.....	2	2	10	2
5	Nuttalliosis.....	1	1	3	—
1	Nuttalliosis and gangrenous pneumonia.....	—	—	1	—
1	Nuttalliosis and horse-sickness?.....	—	1	—	—
1	Nuttalliosis and horse-sickness.....	1	—	—	—
1	Nuttalliosis sequel.....	—	1	—	—
3	Poverty.....	—	—	2	1
1	Malignant oedema.....	1	—	—	—
1	Broncho-pneumonia.....	—	—	1	—
1	Rupture of aorta.....	—	—	1	—
1	Gangrenous pneumonia.....	—	—	1	—
1	Rupture of stomach.....	—	—	1	—
1	Septic enteritis.....	—	1	—	—
1	Gastric impaction.....	—	1	—	—
1	Pulmonary oedema.....	—	1	—	—
1	Colic.....	1	—	—	—
1	Torsion of colon.....	1	—	—	—
1	Toxaemia.....	—	—	1	—
1	Septic infection.....	1	—	—	—
1	Purulent pneumonia.....	—	—	—	1
1	Acute hæmoglobinuria.....	—	1	—	—
1	Syncope.....	1	—	—	—
131		34	44	48	5

(3) *Gelatinous-Marrow.*

No. of Cases.	Post-mortem Diagnosis.	Condition.			
		Good.	Fair.	Poor.	Very Poor.
1	Pulmonary thrombosis and oedema....	—	1	—	—
1	Broncho-pneumonia.....	—	—	—	1
1	Innutrition.....	—	—	1	—
1	Anaemia and debility.....	—	—	1	—
1	Nuttalliosis.....	—	—	1	—
1	Horse-sickness.....	—	—	1	—
6		—	1	4	1

(4) *Gelatinous and Red Marrow.*

No. of Cases.	Post-mortem Diagnosis.	Condition.			
		Good.	Fair.	Poor.	Very Poor.
14	Horse-sickness.....	1	7	6	—
3	Pernicious anaemia.....	—	—	2	1
12	Anaemia.....	—	—	7	5
9	Killed.....	—	1	6	2
5	Nuttalliosis.....	—	—	4	1
2	Glanders.....	—	—	2	—
1	Gangrenous pneumonia.....	—	—	1	—
2	Rupture of colon.....	—	1	1	—
1	Myelitis haemorrhagica dorsalis.....	—	1	—	—
1	Pulmonary thrombosis and congestion..	—	—	1	—
1	Rupture of stomach.....	—	—	1	—
1	Septic pneumonia.....	—	—	1	—
1	Purulent broncho-pneumonia.....	—	—	1	—
1	Colic.....	—	1	—	—
54		1	11	33	9

Summary.

	Total Cases.	Good.	Fair.	Poor.	Very Poor.
Group 1 Fat marrow.....	10	1	5	3	1
„ 2 Fat and red marrow.....	131	34	44	48	5
„ 3 Gelatinous marrow.....	6	—	1	4	1
„ 4 Gelatinous and red marrow....	54	1	11	33	9
	201	36	61	88	16

These results, which have an additional interest lent to them in view of Ackerknecht's findings (Virchow's Archiv. Bd. 208, Heft 3), will thus serve to show that the appearances met with in the bone-marrow of animals dying from pernicious anaemia may also be met with in animals coming to post-mortem as a result of various other conditions, and therefore cannot be regarded as especially characteristic lesions of the former disease.

Other interesting points which these figures also demonstrate will not be specially discussed here, but they will be apparent from a study of the summarized results.

In order now to compare our findings with those of other writers a brief general reference to the literature on the subject is here entered into.

THE DISEASE AS DESCRIBED IN THE LITERATURE.

In discussing the disease as it is described by investigators in other countries it is not our intention to deal with the subject in a

detailed manner, but rather to review it in a somewhat general fashion, and thus bring out only the more salient features which have been noted by those workers who have made a close study of the condition.

The aetiology of the disease having been first definitely established by Vallée and Carré, their names are amongst European workers pre-eminently notable, but detailed investigations have also been made by Ostertag, Hempel, and, later, Seyderhelm, in Germany, and Marek, in Hungary, and other authors have drawn attention to its occurrence in other parts of Europe.

It does not seem to us, however, that the disease described in Switzerland under the name of pernicious anaemia is identical with the disease under discussion.

In America the disease has been investigated by Van Es, Harris, and Schalk, as well as by Mohler, Francis and Marsteller, Mack, and others in the United States, and in Canada, Todd and Wolbach. Rutherford, Torrance, and Watson are amongst those who have studied the condition, whilst in Japan it has received special attention at the hands of a special commission appointed by the Japanese Government in 1909.

As to the aetiology of the disease, workers in all of the above countries where the disease has been described agree in stating that the causal agent is one which is capable of passing through a porcelain filter, and even through one of very finely porous variety. This causal agent is present in the blood and blood-serum of affected animals as well as in the organs, Japanese workers having succeeded in transmitting the disease through the injection of emulsions of various organs taken from affected animals, such as spleen, liver, kidneys, spinal-cord, lymph glands, salivary glands, muscles, lungs, and bone-marrow, and Carré and Vallée having made a similar observation in regard to the first-mentioned of these organs when pursuing their researches at an earlier date. The virus is also to be found in the urine of infected animals, and further is stated to be present in the milk of affected mares and in the blood of newly born foals, this latter fact proving its capability of infecting the animal *in utero* through the placental blood-stream. Its presence in the faeces has also been stated by European workers, but observations in America and Japan fail to confirm this where actual blood is absent. Japanese investigators were also unable to determine the presence of the virus in the sweat, and Ostertag and Hempel failed to infect animals with the saliva from horses suffering from the disease. The Japanese, however, have made one very interesting observation in connection with the distribution of the virus in showing, in the case of two horses in which the virus could not be demonstrated in the blood, that still an extract of their viscera on injection into susceptible animals was capable of producing the disease.

The exact nature of the virus is still a matter of discussion, but recently Seyderhelm has asserted that he has been able to reproduce the disease by the inoculation into susceptible animals of extracts made from the larvae of *Oestrus* species, and claims that the disease produced in this way is serially transmissible from horse to horse.

As to the pathogenicity of the virus, it is stated that the virus obtained from animals in any stage of the disease is capable of producing the affection, and that injection of such virus may produce any of the known forms of the disease. According to Vallée and Carré the quantity of virus injected does not appear to directly affect the

reaction obtained, and one cubic centimetre of virus, they say, may produce as typical a reaction as the injection of one hundred cubic centimetres. In the case of infection by the alimentary tract, however, larger quantities of virus seem necessary to produce the disease than where infection by either intravenous or subcutaneous routes is practised.

The period of incubation is stated as being variable between about five and twenty-five days, but the Japanese observed a period of three days in one case. The American workers state that it may generally vary between six and twenty-one days, and appears to be shortest when intravenous infection is practised, whilst it is longer when the subcutaneous route is used, and longer still if infection by the alimentary tract is attempted, and in this latter case also the incubation period is said to be prolonged further still if urine is used instead of blood for the purpose of inducing the infection.

As to the maximum period for which the virus may remain in the body of an affected animal a definite statement is not made, but that it can there exist over very long periods is a fact which is generally recognized, as is also the fact that animals which appear to have clinically recovered from the disease may still harbour the virus, and thus act as "virus carriers" or "virus reservoirs." Thus European workers state that the blood may still be infective four to five years after infection is first noted, and American investigators state that the blood in one of their cases was still virulent after thirty-five months, the animal concerned not manifesting any clinical evidence of the fact, whilst the Japanese workers found the blood of horses infective three and four, but not five, years after the original infection was produced.

This question of duration of infection thus involving the question of recovery, it may be here noted that European workers believe real recovery to be very rare. The American workers, Van Es, Harris, and Schalk, however, state that recoveries amongst non-anaemic "virus carriers" may be more common than has hitherto been thought, but that it seems that recovery only takes place after a long period during which febrile attacks occur less and less often and finally cease, and the Japanese believe that in non-clinically affected "virus carriers" the virus gradually loses its virulence and ultimately disappears, allowing recovery to take place, their experiments referred to above demonstrating the infectivity of blood of animals after three and four (but not after five) years from the time of infection being brought forward in support of this view.

As regards the transmissibility of the disease to animals other than the horse, infection of the ass is noted in European and Japanese experiments, and the appearance of the disease also in the mule is a fact recorded by American observers. The Japanese workers claim to have also infected the pig with the disease, but outside of this case the disease does not appear to have been transmitted to any animals other than equines, although attempts to do so have been numerous and the species used have included cattle, sheep, goats, dogs, pigs, and the various laboratory small animals.

In reference to the occurrence of the disease and the manner in which natural infection obtains, the European investigators believe that infection is conveyed through the ingestion of food or water contaminated by the excretions of affected animals, and that infection in this way may occur either in the stable or in the pastures, and no particular stress is laid on seasonal or local prevalence of the disease.

Cases have been observed where affected and susceptible animals have been kept in the same stable for long periods without the latter animals contracting the disease, though a rigid isolation may not have been carried out, but even such cases Ostertag explains by remarking that as infection by ingestion usually occurs by the single ingestion of a large quantity of virulent material, or repeated small quantities of the same, that therefore the occasional ingestion of a small amount of contaminated material is not necessarily dangerous.

In America the disease is described as being most frequently associated with low-lying swampy pastures (hence one of its names "swamp fever"), and a seasonal prevalence of the disease is indicated by the number of cases met with in the late summer and early autumn. It is pointed out, however, by Van Es, Harris, and Schalk that it may be also met with under conditions where deficient drainage could not be considered as a factor in its aetiology, but even whilst drawing attention to this fact and recognizing that the disease may be contracted either in the stable or in the pasture these same authors state it as their belief that it is in damp, swampy, or marshy pastures that most infections occur; and they further state that "whilst not denying the possible transmission of the disease to healthy animals by means of insects and parasites, animals contract the disease naturally by the ingestion of food and water contaminated by the urine of an affected horse."

In Japan the seasonal prevalence of the disease is a feature which is well marked, and it there is met with most frequently during the months of summer—when, as the Commissioners say, insect life is at its height—the horses principally affected being those which are running at pasture at that time, and that such a prevalence should be encountered finds a ready explanation if we accept the views of the Japanese investigators in regard to the spread of the disease in that country, for these workers, whilst admitting the possibility of infection by the ingestion of materials contaminated by infected urine, and recognizing that infection in this way does at times occur, do not consider that this is to be regarded as the way in which the disease is principally spread under the conditions obtaining in Japan, but on the other hand have come to the conclusion that the chief manner in which transmission under natural conditions must be considered to occur is through the agency of some flying insect or insects of blood-sucking habits.

It must be stated, however, that at the present time this belief has not found direct experimental verification and rests only on indirect evidence gained from certain ingenious experiments which were performed to test its correctness, but, so convinced are the Japanese Commissioners that this is the way in which the disease is spread in their country that in their official regulations for dealing with the disease we find a definite statement made to that effect, and they go even further than this in suspecting that the insects concerned are to be found amongst the flies of the Tabanidae group, other insects—and also certain species of ticks—being excluded from suspicion for various reasons, which in their report will be found mentioned in detail.

The symptomatology of the disease has been well described by numerous writers, but we shall here briefly review only some of the descriptions given, referring first to the now classical description of

the disease as occurring in Europe, given by Carré and Vallée, who described it in an acute, sub-acute, and a chronic form.

In the acute form the disease is said to appear rather suddenly, being primarily evidenced by the animal having a rather dejected dull appearance, being lazy, and tiring easily when working. If work is forced, the exertion causes an increase in respirations, associated with the appearance of weakness in the hindquarters, and this latter condition causes the animal to assume a swaying uncertain gait and trail the hind feet along the ground. If turned suddenly the animal may fall, and may then have great difficulty in rising owing to the paresis of the muscles of the hindquarters. Some of the positions assumed by the animal when resting in the stable are described as suggesting that it may be affected with tetanus, whilst at other times laminitis is said to be suggested owing to the manner of walking and standing. The appetite is decreased, and the animal may take food into the mouth, but show no inclination to masticate or swallow it. The pulse rate may be as frequent as 60-90 per minute. The heart sounds are accentuated in character and of a more vibratory character than usual, and very slight movement is said to be sufficient to cause the pulse to become irregular and intermittent. The temperature gradually rises and reaches its maximum, which is usually 104° F. or 105° F., in a few days. It may ascend as high as 107.5° and remain high until the point of death, but at times remissions of twenty-four to forty-eight hours may be noted. The conjunctiva shows injection of the vessels and is oedematous. Its colour may be reddish or reddish-yellow, and on this background may often be noted petechiae of a colour varying from pink red to brown or purple. Lachrymation and photophobia may also be present. There may be evidence of slight oedema of the more dependent parts of the body. The urine is said to be abundant and of a high colour (deep brownish yellow), but hæmoglobin and red corpuscles are absent. The presence of albumin is said to be constant and stated by Vallée and Carré as being 14 grammes to the litre, and epithelial and granular casts may be met with. The faeces may be reddish in colour and thus suggest the presence of blood, though spectroscopic examination proves its absence, but in some cases where symptoms of colic and enteritis are present the faeces may then be streaked with it. It is noted that this form of the disease often brings about abortion in pregnant mares, and the usual termination is met with in death, which event may occur inside a period varying from 5 to 15 days, and is usually preceded by rapid emaciation as well as a paralysis of the hindquarters, which latter is evidenced as the end approaches by inability to rise, incontinence of urine, and relaxation of the anal sphincter.

In the sub-acute form the symptoms are somewhat similar, but are modified and associated in different ways, depending on the stage of the disease present. The chief characteristic of this form of the disease is the appearance of clearly defined remissions of the fever, which, in the absence of anaemia, might cause one to believe that the animal had recovered. In these cases, however, examination of the urine still proved the presence of albumin, and the blood is said to have revealed a marked anaemia, whilst the conjunctiva presented an oedematous appearance. Associated with the remissions of the fever there was also observed an amelioration of the symptoms in connection with the pulse, respiration, the oedema of dependent parts, and the general dullness shown by the animal when passing through the

febrile reaction. This form of the disease may last for a period of weeks or months, and may then pass into the chronic form, or death may occur from the exhaustion produced by the repeated exacerbations of the disease. Death may also occur during one of the exacerbation phases, and such phases are sometimes determined through the forced working of the animal which, whilst at rest, may appear as if recovered, but when worked then usually very quickly shows signs of distress, perspiring profusely, with cardiac action increased and laboured, and ultimately falls to the ground exhausted by its efforts.

The chronic form is evidenced by the existence of a markedly anaemic condition which is insidious in onset and progressive in character. The animal becomes gradually poorer and weaker in condition, and shows a staring coat, and the visible mucous membranes present a pale and anaemic appearance, with sometimes a pale yellow tinge apparent. Oedema of the dependent parts of the body is seen, and the uncertain gait produced by weakness of the hind-quarters is present. The pulse is soft, frequent, and unequal, the artery being easily compressed and "flabby." Cardiac irregularities in the form of intermittency, palpitation, and unusual cardiac sounds are to be noted, and sometimes a jugular pulse is apparent. The temperature in this form of the disease remains for the greater time normal, but intermittent febrile attacks occur from time to time. The appetite is said to be capricious, and sometimes a diarrhoea of transitory character is noted. Urination is frequent, and the urine is present in large quantities and generally contains albumin. If the animal is worked it is very easily distressed and very soon exhausted. This type of the disease can allow of the existence of the affected animal for long periods, extending maybe into many months, and death may either occur during one of the interfebrile periods, and as a result of extreme anaemia and debility, or, on the other hand, may occur during one of the remissions of the fever.

Changes occurring in the blood are also referred to in dealing with the different forms of the disease described. In the acute form the blood is said not to coagulate well, and the serum, too, often has a yellowish green tint. The red corpuscles after 10 to 15 days may have fallen one or two millions below normal (7,000,000 C. and V.), and just before death may only number about 4,000,000. They are said to be brownish in colour, to agglutinate easily so as to form small masses which float in the plasma before setting, and to be friable, hæmoglobin poor, and varying in size. It is also said that a larger number of them showed punctations and appeared as if containing intracorpuseular bodies. The absolute leucocyte count is said to be usually 1000 or 1500 per cm. less than normal, a polynuclear hyper leucocytosis being, however, present.

In sub-acute cases and towards death the red count, it is said, may fall to 2 or $2\frac{1}{2}$ millions.

In the chronic form the blood does not coagulate well, and the red cell count may vary from 2 to 4 millions, and may fall as low as 1 million. In this form there may also be a slight absolute hypoleucocytosis, and the mononuclear and polynuclear cells be equal in number.

Hutyra and Marek also describe the disease, but only recognize two clinical forms, namely acute and chronic, between which no sharp line is drawn. The symptoms in the main correspond to those mentioned in the description given by Vallée and Carré. but the former

authors state that the acute form may, instead of only lasting 5 to 15 days, only prove fatal for adults in three to four weeks, though young foals, on the other hand, may die in 1 or 2 days. The duration of the chronic form they state as being sometimes one to several months, and not infrequently several years.

The disease as it occurs naturally in the field has been described by many writers in America, but here we will only refer to the remarks of Van Es, Harris, and Schalk, who have made a careful study of the disease both in the field and under experimental conditions.

These writers state that fever is perhaps the most constant feature mentioned in connection with the disease, and that in the field cases it is always more or less irregularly remittent or intermittent, ranging between 101° F. and 104° F., with a tendency at times, especially towards the end, to remain more constant, though this feature is not of regular occurrence. They also state that the pulse is often influenced by the temperatures, and that in anaemic field cases of long standing the pulse is soft and thready, whilst in more vigorous experimental animals it is more voluminous and differs little from the normal, save in frequency. In all cases, save possibly vigorous experimental animals, the pulse frequency varies between 55 and 65 per minute, and may even reach 90 or 100, or more towards death, becoming at the same time smaller, weaker, and at last imperceptible. In the latter stages of the disease changes of cardiac origin were noted, but no specific respiratory changes were met with. In some cases dullness of the animals was noted, but in not a few cases the febrile symptoms were unaccompanied by any special symptoms save a slight increase in the respirations. During the febrile attacks the appetite was reduced or absent, but in many chronic and anaemic cases a voracious appetite was present. Weakness and loss of muscular tone is also a symptom occurring in anaemic and non-anaemic cases, and may often be marked, the animals on exercise readily tiring and blowing and showing an inclination to "knuckle-over" and stumble, or presenting a swaying "wabbling" movement of the hindquarters. Loss of flesh is said to usually accompany the febrile attacks and is often progressive, emaciation being more or less constant when anaemia is marked. Associated with loss of condition is the appearance of a dull staring coat and hide-bound condition, and oedema of dependent parts was noted commonly in later stages of anaemic cases, but was also seen in acute experimental cases in which anaemia was not a feature. Anaemia was more or less marked in all the field cases save one, but in experimental cases was by no means constant, and was only present in marked degree in a few cases, several animals dying with a blood count which was normal or even slightly above the normal. They found that albuminuria was frequent in both field and experimental cases, but was not constant, and in a given case might be transitory, and it was never present in the degree noted by European authors. In some cases they say they found a sharp well-defined albumin reaction, but in the greater number of cases there was only a trace to be found, and in only one case were tube casts present. Polyuria they state as being occasionally seen, and then more particularly towards the end in chronic anaemic field cases.

We will not here enter further into the description of the disease given by these workers, as the above notes will suffice for our present purpose, and it does not appear necessary to refer in detail to the

description given by the Japanese workers, since the description furnished by these latter closely resembles the general symptoms of the disease described both in Europe and America.

In reviewing the post-mortem appearances which, similar to the symptoms, have also been described by a number of observers, no description of changes specific to the disease are met with, although a certain amount of stress is laid on the appearance of the bone-marrow, and by European authorities in particular. The description of the post-mortem appearances given by Hutyra and Marek may be quoted as a general description of changes which may be found, but other authors also give good descriptions of the changes found at autopsy, and Seyderhelm has recently given a good description of the histo-pathology of the disease. Hutyra and Marek state that the disease is characterized by anatomical changes of an acute or chronic septicaemia, with visible changes in the blood, and in which the changes, depending on the duration of the disease, may show very pronounced variations. The spleen is described in animals dying from the acute form or during a relapse as enlarged to maybe twice or four times its normal size and with tense capsule covered with hæmorrhages, the pulp being soft, mushy, or even liquefied, as in anthrax. Sometimes the organ may show nodulated protuberances, marking the presence in the pulp of softened black-red areas, and here the spleen may be otherwise normal, or more or less enlarged. In cases where the course of the disease is slow they state that the swelling may not be marked, and in chronic cases it is said that it may be altogether absent, the consistency being then, at the same time, increased. The lymph-glands may be swollen and show hæmorrhages in cases where these latter are also to be found in the corresponding organs, but in chronic cases the glands may show little or no swelling. Subserous hæmorrhages are usual, except in chronic cases, and when present may be found in various situations. In the peritoneal cavity they may be especially numerous under the serous membranes of the colon and cœcum, and may also be met with under the capsule of the liver and spleen. They are usually found in the heart, except in chronic cases, and vary in size and number with the intensity and duration of the disease, the heart in very acute cases appearing as if sprinkled with blood. Hæmorrhages may also be met with in the gastric mucosa and also in the mucous membrane of the intestines, being either single and rounded or occurring in the form of large diffuse patches, and in some cases the intestinal contents may even be blood-stained. They may also occur in the mucosa of the bladder, and, in acute cases, may be met with in the lungs and kidneys. The liver is more or less enlarged and pale yellowish, and may be very friable and, in company with the heart and kidneys, show parenchymatous degeneration. There may be a small quantity of yellowish, or reddish-yellow, serous fluid in the peritoneal cavities. In acute cases the blood shows no peculiar macroscopical changes, but in chronic cases there are indications of anaemia. Marked emaciation is often seen, and it is also mentioned that oedematous infiltrations of the subcutaneous, intermuscular, and perirenal connective tissue, as well as of that between the mesenteric layers, is to be met with, whilst the presence of icterus in some cases is also noted in addition.

Certain appearances presented by the bone-marrow are also described, but these appearances have already been alluded to in another part of this article.

In regard to the diagnosis of the disease, the difficulty of determining its presence or otherwise is a difficulty of general recognition, and no really satisfactory method of diagnosis outside of the costly procedure of inoculation of a susceptible horse has yet been established. The examination of the blood, either quantitatively or qualitatively, and morphologically, physically, or chemically, does not furnish sufficient evidence to allow of an absolute specific diagnosis being made, and sero-diagnostic methods so far applied have not up to the present time proved successful. Diagnosis by the complement-fixation method failed in the hands of European, American, and Japanese investigators, and the search for specific hæmolysins, either iso- or auto-lysin, has similarly not met with success, whilst Japanese workers have also found the precipitin and cuorin reactions not to be of any service, and though Abderhalden and Frei, and Abderhalden and Buchal, have stated that the serum of infected animals inhibits the hæmolytic action of saponin and snake venom upon the red-corpuscles, yet they have not advanced their researches far enough to test the practical value of the diagnostic methods suggested by this fact.

Hutyra and Marek state that in infected localities or stables the clinical symptoms, course, and negative findings in various organs usually suffice to establish a diagnosis, but that when on the contrary the locality has been previously regarded as free from the disease, the diagnosis can then only be recognized with certainty on autopsy or animal inoculation, the clinical symptoms arousing only well-founded suspicions. They state also that the special indications which are to be found post-mortem are changes of a septicaemic character, such as hæmorrhages into serous or mucous membranes, further the regular discoloration of the marrow of the bones of the upper extremities, degeneration of the parenchymatous organs, swelling of the spleen, and cedematous infiltrations without other changes in the organs.

Ostertag and others have laid down certain rules for guidance in diagnosis, and state that the disease can be suspected in a horse in which there is noted dullness, poor condition, pale or pale-red mucous membranes, decrease in the red-corpuscles (estimated in the field by Zschokke's method) and increase in pulse frequency, this latter being markedly brought out by very slight movement. To these symptoms may be added fever, oedema, and excretion of albumin with the urine.

The diagnosis is regarded as assured when, on post-mortem examination of an animal showing the above symptoms, the changes indicative of a septicaemia are met with, such as cloudy swelling of liver, heart, and kidneys, enlargement of the spleen and lymphatic glands and subserous hæmorrhages, and, in addition, subcutaneous oedema and hæmorrhagic foci in the fat-marrow of the tubular bones.

Mohler says that diagnosis is not difficult, especially in advanced stages, and relies on such characters as the insidious onset, remittent fever, progressive emaciation, unimpaired appetite, staggering gait, and polyuria to differentiate it from other diseases affecting horses in America, and says that the great red-blood cell reduction but absence of eosinophilia differentiate it from anaemia due to internal parasites.

The Japanese state that during febrile exacerbations one can easily diagnose the disease from the symptoms, but that in the

chronic cases with normal temperature, and during interfebrile periods, diagnosis is difficult or impossible, and only to be inferred from emaciation, alterations of the mucous membranes, and a peculiar change in the cardiac sounds, and that the only sure method of diagnosis in these cases is the inoculation of blood into a susceptible horse.

Van Es, Harris, and Schalk also remark on the difficulties of diagnosis, but state that diagnosis in the anaemic type of the disease is often possible from the symptoms and history of the case. They regard an anaemic fever case with occasional albuminuria, oedema, and a swaying gait as suspicious, and if in addition to this there is a history of several previous cases of the same nature, and this in a swamp fever locality, they then regard a diagnosis of swamp fever as justifiable. They dwell, however, upon the difficulty of diagnosing the disease in the non-clinically affected "virus carriers," and suggest that outside of blood-inoculation the only way to recognize these cases is to systematically temperature all suspected animals for long consecutive periods. Even then, however, they say, some of these animals may show no febrile reactions, and although some may do so, it may not in these cases be possible to definitely lay it down as being a swamp fever reaction, and they further remark that whilst the occurrence of albuminuria might here furnish help, yet this symptom might well occur also in other diseases.

In referring to the differential diagnosis Hutyrá and Marek make mention of—

- (1) The catarrhal form of influenza, and differentiate this from the acute form of infective anaemia on the fact that the former disease spreads rapidly in the stable; that the course is always rapid and favourable, and that catarrh of the mucous membranes prevails whilst hæmorrhages are absent.
- (2) Anthrax, and differentiate it from the acute form of the disease by the rapid rise in temperature met with in anthrax, as well as the not infrequent appearance of circumscribed rapidly extending inflammatory oedemas, associated with colicky pains, cyanotic conjunctiva, dyspnoea and a duration of about one to three days, further stating that if death only occurs after ten days anthrax may be excluded by this fact alone.
- (3) Enzootic cerebro-spinal meningitis, and in this case they state that differentiation can only be established through inoculation.
- (4) Equine piroplasmosis, and this they state is to be differentiated through the icteric discoloration of the membranes in this disease associated with the demonstration of parasites in the blood, and its non-inoculability to healthy animals. (This last being apparently a misstatement.)
- (5) Sclerostomiasis in its chronic form, and this they state is to be recognized by running an afebrile course and attacking young animals exclusively, whilst the acute form of the same condition may be recognized by the finding at autopsy of sclerostome larvae in the intra-abdominal hæmorrhages, care being taken not to be misled by seeing a few larvae in the subserous or submucous tissues,

- (6) Simple anaemia, and this is differentiated by its afebrile course, presence of some primary cause, dietetic errors, or the existence of some outside factor.

Leaving the question of diagnosis and next passing to consider the views in regard to possibility of cure we note that attempts to procure a curative treatment for the disease have been very numerous, but so far have not resulted in any specific curative agent being discovered, nor do any of the drugs tested appear to have exercised any lasting beneficial effects in producing recovery from the disease, the treatments adopted proving to be only palliative and not curative. Amongst the various medicinal agents used in attempts to discover a curative are mentioned such drugs as quinine salts, arsenic and arsenical compounds, e.g. atoxyl, salvarsan, and arsenophenylglycin, also potassium sulphate, potassium and sodium bicarbonates, nuxvomica, strychnine, sulphate of iron, carbonate of iron, iodine, mercury, Collargol, Peru-balsam, salol, salicylate of sodium, cacodylate of sodium, cacodylate of iron, thymol, thiokol, ichthyol, mercuriol, liquor cresol, antimony sulphate, veratrine, anti-febrine, antipyrine, phenacetine, trypan-blue, trypan-red, lecithin, cholesterin, and glycerine extract of bone-marrow.

As a palliative treatment, Van Es, Harris, and Schalk speak of the use of arsenic and iron carbonate combined with gastric stimulants, good food, and careful attention to general hygienic conditions.

Similarly, attempts to obtain a prophylactic or curative serum have also failed. Vallée and Carré thought that serum derived from horses apparently recovered, and subsequently injected with large quantities of virulent blood, or the serum of asses or cattle injected with virulent blood, might be of service in this respect, but the results obtained were not encouraging, and Hutyra and Marek state that the same must be said of Marek's experiments made with the blood of an apparently recovered horse, as well as with blood subjected to the action of various high temperatures or to trichloride of iron solution. The American workers state that they made too few experiments in this direction to justify definite judgment on the question, but their experiments, made in injecting blood of a hyperimmunized animal combined with the injection of virulent blood, gave unsatisfactory results, as did also similar experiments made by the Japanese. These latter workers also carried out experiments in using for inoculation the virus heated to 60° C. for one hour, associated with the injection of serum derived from goats inoculated on several occasions with virulent blood, but here, again, the results obtained were also negative. Serum and bile derived from a calf inoculated with virulent blood were also useless for immunization purposes. A pig was inoculated with virulent serum and its blood taken on the third day following the inoculation and also a month later was injected into susceptible horses, but these horses, however, died as a result, and this fact is interpreted as showing that the virus was not attenuated by passage through the pig. They also made experiments with "immune-serum" rendered sterile either by heating to 60° C. for one hour, or by means of 0.5 per cent. tritresol, but experiments with this serum, including one where horses were injected once a month throughout the pasturing season, were also negative.

Preventive treatment is laid down by most authors along generally similar lines, with the exception, perhaps, of the Japanese

who, on account of their views in regard to transmission, pay more attention than any other authors to measures directed against the attacks of biting insects.

In preventing the introduction of the disease into clean areas, the greatest care is advised in the purchase of new horses, particular attention being directed to be paid to the district they come from, and all authorities lay stress on the fact that the great source of danger, even where care is taken in attempting to prevent the introduction of the disease lies in the existence of horses which, though apparently recovered, are still virus-reservoirs. Vallée and Carré recommend that horses from suspected districts should be quarantined for at least a month before being placed in a common stable with the horses already on the premises, and that the urine should be examined for albumin as this is always present in larger or smaller quantity, and that the condition of the heart in relation to exercise should also receive attention in making a diagnosis. Ostertag recommends that the newly introduced horse or horses should be stabled separately from horses already on the premises, and suggests the cattle stable for this purpose. Where this isolation cannot be effected and they have to be stabled with the other horses, he states that care must be taken to place them in stalls where they are well partitioned off from their neighbours, so that in this way, as well as through the care of attendants, the hay or straw used for the bedding or feeding of the general horses may not be contaminated by the excreta of the newly introduced animals. It is also necessary that the newly introduced animals should not be watered at the general trough, but separately from the other horses, and the continuance of these measures for a period of three months following the introduction of new animals is recommended on account of the insidious nature of the disease.

Hutyra and Marek state that suspected horses, and especially those which without apparent cause show signs of poor condition, anaemia, increase in frequency of cardiac action after an insignificant amount of exercise, are easily fatigued and show the presence of albumin in the urine, should not be admitted to the general stable until it is determined that they are free from infection. This latter involves isolation and observation of the animals for a period of three months, and for isolation purposes the cattle stable is by these authors also suggested.

The American investigators suggest that restriction should be placed on movements within and out of infected areas, and suggest that at least during the part of the year from about July to December movement of horses should, in infected districts, be entirely suspended, as this is apparently the season when most actual transmissions occur. They also recommend that suspected animals, even though appearing to recover, should not be moved from place to place as they may be virus-carriers, and they advise farmers living in infected districts and having non-infected stables, that if they wish to introduce new animals these should be purchased from another and clean district. In dealing with an outbreak of the disease in a stable or on a pasture, the following recommendations are made. The affected animals should be isolated, preferably by leaving affected animals in the stable where they stand and removing the healthy horses, and on no account should the affected animals be sent to pasture. It is also suggested that

affected animals should preferably be destroyed, but if they are kept another place which is suggested for isolation is again the cattle stable. Hutyra and Marek recommended that when the disease occurs on the pasture the affected animals should be isolated and stabled and the healthy horses removed to a fresh pasture not used before for horses.

Disinfection of stables is also to be carried out as well as the disinfection of excreta of affected animals, and Ostertag recommends for the latter purpose the packing of excreta for a month in heaps of a cubic metre in size.

Water supply is a matter also generally referred to, and a supply uncontaminated either directly or indirectly by the excreta of affected animals is insisted upon, a great source of danger to be here guarded against being said to exist in the presence of shallow wells or pools receiving the drainage from stables, manure heaps, or cesspools, as well as in ponds, pools, or ditch water on marshy or swampy pastures.

It is also pointed out that attention must be paid to the food and litter supplied to healthy horses to see that this shall not become contaminated by infective excreta either directly or indirectly through the carelessness of attendants.

The construction of hygienic stables with impermeable flooring which allows of a thorough disinfection, and which may also reduce the risk of contamination by drainage of neighbouring water supplies is also advocated, and the American investigators also recommend the proper drainage of swampy or marshy pastures and the protection of animals against the bites of blood-sucking insects, regarding these latter as being possible transmitting agents of the disease.

In Japan the prevention of the disease is dealt with under the "Ordinances applying to the Prevention of Infectious Diseases of the Domestic Animals," and in the description of the disease embodied in the "Notifications" of these Ordinances it is stated that "the disease is of an infectious nature, the virus being mainly transmitted by horse flies. It spreads in the pasturing districts, and infection rarely takes place in the stable or when the horses are employed for work." Various measures for the detection of affected animals are detailed, and it is stated that these affected or suspicious cases may be isolated or may have to be destroyed. Rules regarding the pasturing and movement of animals in declared infected areas or "prevention districts" are also laid down. Isolation of positive and suspicious cases when practised must be carried out in stables so constructed as to cut off direct sunlight and thus prevent the swarming of horse flies, and if a positive case occurs amongst a pastured troop during the horse-fly season the whole troop is regarded as infected and kept under observation for the whole pasturing season. Healthy horses working near infected pastures must not be worked, they state, in direct sunlight, owing to the swarming of horse flies.

In regard to stables they state that these must be made clean, and lay down that "the floor must be washed either with hot alkali or water. The earth under the floor must be removed by digging more than six inches deep and the hollow filled with clean soil. The floor straw, droppings, and the earth containing dung and urine that have been kept in heaps for two months may be used as manure."

The destruction of positive cases of the disease is advised.

THE PROBLEM INTRODUCED BY THE DISEASE INTO THE PRODUCTION OF HORSE-SICKNESS ANTI-SERUM, AND METHODS USED FOR DIAGNOSIS OF THE DISEASE.

The above review of the literature, brief as it is, has however shown the similarity existing between the disease we describe and that described under such names as infectious or pernicious anaemia of equines, swamp fever of horses, etc., by authors in other countries, and this being so we may now refer to the problem which the presence of this disease presents in the production of horse-sickness anti-serum.

The method of preparation of this serum has already been referred to in the earlier part of this article, but may again be noted here as consisting essentially of a "hyperimmunization" of horses previously immunized against the disease ("serum" horses) by means of an intra-jugular transfusion of large quantities of virulent blood (10 litres) derived from horses ("virus" horses) which are passing through a horse-sickness reaction induced by injection with horse-sickness virus.

This, therefore, will allow it to be seen at once that the greatest care has to be exercised in the selection of "serum" and "virus" horses, as well as of the materials with which they are injected in order to ensure the absence of the virus of infectious anaemia from the serum ultimately obtained in bleeding the "serum" horse.

In the detection of the disease, however, we have encountered the same difficulties as other writers describe, and it must be said that the recognition of the disease is often a matter of extreme difficulty if inoculation of blood of a suspected case into a susceptible animal be not practised.

The differentiation of the disease from the other two diseases common in equines in South Africa, namely, horse-sickness and biliary fever or Nuttalliosis (equine piroplasmosis) is not a matter of great difficulty where typical and uncomplicated cases of the latter diseases are met with.

Thus in cases of horse-sickness where the typical symptoms of the "dikkop" form of the disease are present it is not likely that mistakes will arise, and it is also unlikely that the disease will be confused with cases of "dunkop" horse-sickness showing well-marked symptoms of respiratory disturbance, increased pulse, injection of mucous membranes, and, towards the end, discharge of yellowish liquid or yellow-white foam from the nostrils.

On the other hand, however, and under experimental conditions, one does meet with cases where, following injection of horse-sickness virus and depending apparently on the particular strain of virus used and the degree of immunity possessed by the animal injected, febrile reactions are sometimes encountered which at first offer a certain amount of difficulty in diagnosis. In these instances, however, the further progress of the case and the subsequent presence or absence of repeated febrile attacks and other symptoms of the sub-acute or chronic forms of infectious anaemia will serve to allow of the ultimate differentiation.

The differentiation of infectious anaemia from the disease described elsewhere by one of us (Sir A. T.) as ephemeral fever can also be made along similar lines.

That it is experimentally possible to separate the virus of infectious anaemia from that of horse-sickness when both these filterable viruses are present in the one sample of blood, and this by the injection

with such material of horses immune to horse-sickness, is a matter which has already been mentioned being dealt with in this article in describing the manner in which we first came to recognize the existence of the former disease in equines in this country.

In the case of Nuttalliosis (biliary fever, equine piroplasmosis) it is also not difficult to differentiate it from infectious anaemia in those cases where, in addition to dullness and languor, loss of appetite, high temperature, soft pulse of increased frequency (80 to 100 or more per minute), there is also present well-marked icteric discoloration of the membranes, the appearance of the causal parasite (*N. equi*) in the blood, and possibly hæmoglobinuria.

Difficulty in differentiation may, however, arise in those less acute or less typical cases of Nuttalliosis where a dirty-yellow or perhaps pale appearance of the mucous membranes is more in evidence than a bright-yellow icteric discoloration, and the causal parasite (*N. equi*) is rare in occurrence. The ultimate differentiation of cases such as these may involve at times a prolonged observation with regular tempering and frequent blood examination, and it is the difficulty experienced in the differential diagnosis of such cases that leads us to believe that in the past certain cases of Nuttalliosis and infectious anaemia may well have been confounded with one another under the rather collective term of "biliary fever." At the present time, therefore, and with all of these difficulties in diagnosis present, the method upon which we have to place chief reliance for the primary detection of the disease in horses used in serum production is the regular and systematic tempering of these animals.

The "virus" horse, therefore, is regularly tempered (morning and evening) from the time of entering the station, and whereas previously it was the custom to utilize these animals at any time after they had passed the mallein and complement-fixation tests for glanders, they are now kept under observation for from four to six weeks or longer, and any of them which during this time show suspicious temperature reactions—in the absence of definite symptoms of any other disease—are then rejected and utilized for other purposes.

Similarly the "serum" horse is also kept under temperature observations during the whole time it is used in serum production, and whereas formerly this observation was only carried out up to the time of the last bleeding following any given hyperimmunization and then suspended up to the time of the next, it is now carried out during the whole interval separating any two given hyperimmunizations as well as up to about six or seven weeks following the time when the last operation of this nature is performed.

At the same time the serum obtained from individual "serum" horses is kept in separate vessels for several months before mixing, and should any "serum" horse during the above-mentioned regular tempering show a suspicious reaction following hyperimmunization, the blood of such an animal, or the serum previously obtained from it, is injected into a susceptible horse, and if the disease now appears in this latter animal then the serum previously obtained from this animal—whose blood is now shown to be capable of producing the disease—is naturally rejected and later destroyed. If a number of "serum" horses show suspicious reactions a collective test is first made by injecting a mixture of the suspected sera into a susceptible horse, and if the result of this test should prove to be negative no further test is performed. If, however, this collective test proves to

be positive then further tests are carried out in order to isolate the individual infective sera or serum.

It is not necessary to dwell here on the increased cost in serum production which all of these procedures involve, but it may be noted that, whereas previously the number of horses bled for serum was usually 67 per cent. of those entering hyperimmunization (the other 33 per cent. including animals yielding serum haemolytic through the presence of isolysines, as well as those dying from "shock" during the intrajugular transfusion), this percentage must now be reduced somewhat further owing to the rejection of animals affected with infectious anaemia.

As to the distribution and occurrence of the disease in the field little is yet known owing to its comparatively recent recognition in this country, but it is hoped that further investigations will clear up these points and also throw considerable light on the manner in which the disease is most commonly spread in South Africa.

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INVESTIGATIONS
INTO THE
Life-History of the Wire-Worm
in Ostriches.

BY SIR ARNOLD THEILER, K.C.M.G.,
Director of Veterinary Research,

AND

W. ROBERTSON,
Veterinary Research Laboratory, Grahamstown.

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THE object of these investigations was a practical one of placing the preventive and curative treatment of wire-worm infection on a reliable basis. A bare description of the wire-worm was the only information at our disposal at the beginning of our undertaking. We found, however, a most useful guide in the classical work of Looss on the *Ankylostoma* of man, and indeed as far as the anatomical and histological side is concerned the description of the former applies *mutatis mutandis* to the latter, even in many details. Our time did not permit us to enter so thoroughly in these details and we kept more to the morphological structure in so far as it was necessary to differentiate the various stages.

Our main attention was devoted to the biological side of the question, always having in view the practical issues observed under the conditions of the field. In order to trace the life-history and describe the various stages it was necessary to have non-infected chicks which we had to breed ourselves; we succeeded in keeping them free from all parasites in the manner indicated in a later report of this paper. This successful breeding may be considered to be the proof of the correctness of our deductions and will undoubtedly be useful to the ostrich breeder. We regret, however, we are not able to hold out hopes of any absolute cure, viz., of a drug which will remove the worm from the stomach. The reader will understand this when he has gone through this report. We hope, however, to have pointed out the way in which success in ostrich breeding can be attained, and, as will be seen, it leads to a closer domestication of the ostrich.

THE WIRE-WORM OF THE OSTRICH.

Trichostrongylus douglasi (Cobbold).

Synonyms: Haarworm, or Draadworm of the Boers.

Historic Review.

The first description was by T. Spencer Cobbold in the year 1881 (Linnean Society, Vol. XVI), made from material obtained from an experienced and well known ostrich farmer, the Honourable Arthur Douglas, of Heatherton Towers, Grahamstown. It was in honour of this gentleman that the worm received its specific name, after having been recognized by Cobbold that it belonged to the genus *Strongylus*, which at that time

was more comprehensive than to-day. As a new species it had already been recognized by Dr. Bekker, of Grahamstown, a medical practitioner and keen student of nature, to whom the same worm had been submitted for examination.

For the diagnosis Cobbold gave the following characters :

"*Strongylus douglasi*, sp. nov.—Body smooth, transversely striated, nearly uniform in thickness, rather suddenly narrowed in front; head minute, often spirally folded inwards; mouth simple, unarmed, oesophagus long, gradually thickening below; tail of the male with a broad, two-lobed hood and simple ray-arrangement; spicules short, stout, closely applied; tail of the female directed inwards, suddenly narrowing below the anus, which is sub-terminal.

"Length of the male 1-6th of an inch (4·2 mm.), breadth about 1-240th of an inch (0·105 mm.).

"Length of female 1-5th of an inch (5·1 mm.), breadth about 1-215th of an inch (0·12 mm.)."

This diagnosis permits us to place the ostrich wire-worm into the family of the Strongylidae (Cobbold). Since Cobbold's time this family has been split up in a number of genera, and hitherto no attempt has been made to place the ostrich wire-worm into one of the new genera.

Of the text-books on Parasitology, only Gedoelst mentions it in his synopsis of parasites found in domesticated animals as *sedis incertae*, whilst the most modern parasitological book (Fiebiger) does not know of it at all.

It is our intention to give a more detailed description and then to find a place for it in the modern system of parasitic nematodes.

We collected the material principally from birds, killed for the purpose, or on account of accidents.

For the preservation the method of Looss was made use of by placing the worm in a mixture of 2-10 per cent. glycerine in 75 per cent. alcohol, heated near boiling point, and examining it in the mixture, or in glycerine gelatine.

Some discrepancies will be noted when comparing our description with that of Cobbold. They find an explanation in the fact that the material examined by this gentleman had been preserved in spirits of wine.

Size and Form. (Figs. 9 and 10.)

The form is cylindrical, the body has nearly everywhere the same thickness. It tapers slightly and somewhat suddenly towards the head from the region of the oesophageal bulb. In the male the tail end is slightly curved, and the worm in its posterior portion slightly twisted longitudinally. The thickness measured in a male subject gave the following results :—

At the head.....	25μ.
In the region of the oesophageal bulb....	76μ.
In the middle of the body.....	93μ.
Immediately in front of the bursa.....	94·5μ.

The average length of the body amounts to 4650μ . The female is about 1 mm. longer, its average length measuring 5630μ . It is also slightly thicker: the measurements at various places are as follows:—

Head.....	27 μ .
Region of oesophageal bulbus.....	88 μ .
Middle of the body.....	105 μ .
Vulva.....	108·5 μ .
Region of the anus.....	34·5 μ .

In the female from the anus backwards the body tapers to the tail, terminating with a blunt point about 80μ in length; the tip is slightly curved.

The colour of the worm, observed in its natural habitat on the mucosa of the proventriculus, where it is found in clusters, is yellowish-red. An individual worm taken under the microscope appears colourless and transparent. The same holds good when preserved in glycerine gelatine.

The skin is about 3μ thick in the anterior portion and about double as thick in the posterior one. The surface is ringed, the distance between the rings averaging $2\cdot51\mu$. The rings are distinct in the middle of the body, but less so on both ends. The surface (cuticle) is smooth.

Of the four *longitudinal bands*, three are distinctly visible, viz., the two laterals and the ventral one. The lateral bands begin immediately behind the mouth, starting indistinctly with an oval-shaped enlargement, further backwards they stand out clearly and sharply defined; cellular nuclei are visible at certain intervals. These nuclei are large, oval and flat, and contain one or two nucleoli. They are placed in double rows, but not in a regular sequence; in one instance two nuclei may be found opposite each other, in another in alternative positions, and thirdly nuclei may follow one behind the other alongside one margin of the band, to be succeeded by a similar group on the opposite side. In the male these bands, with a width of $30\text{--}32\mu$, end in the bursa; in the female they taper out into the tail. Observed on a cross-section they are raised into the body cavity.

At the head end, squeezed into the course of the lateral band, the cephalic gland may be discerned. This at least is concluded by comparison with a picture given by Looss of *Ankylostoma*. The ventral band is narrow, the nuclei are also arranged in a double row, and placed at short intervals, but are much smaller. In front of the anus this band widens out a little, bordering the anus and forming behind the pulvillus post-analis.

Under the transparent cuticula, longitudinally striated muscles of a drawn out rhomboidal shape become visible, of which four are found on a quadrant across the head end of the body. The nuclei of the muscles are large, oval shaped and flattened. Nuclei of corresponding muscles symmetrically situated are placed more or less in the same cross plane. On cross-section the nucleus is found to be placed in the inner not striated portion of the muscle. Generally speaking the portion of the muscles bordering on the subcuticle is well defined. Occasionally protoplasmic protrusions shoot off from the lateral margins. Particularly prominent are the muscles in the tail end of the male connected with the bursa and

the spicula. Of the latter, two retractors stand out well, one on each spicule having a straight longitudinal direction, whilst the two extensor muscles are less conspicuous. The muscle attached to the outside wall of the rays of the bursa—the retractors of the bursa—and those attached to the inside constrictors of the bursa are distinct and can be recognized individually. The end portion of the chyle intestine is surrounded by a reticulum of fibres corresponding to the muscoli intestinales. In the female is a fan shaped muscle spreading out from the rectum to the dorsal wall.

At the anterior end, alongside the lateral band are superficial muscles attached just behind the mouth; they taper out backwards, and in shape are longitudinally triangular. They apparently affect in particular the movements of the head. The head measures $20\text{--}27\mu$ in diameter, and is circular. The mouth opening, seen from the frontal side, is triradiated, thus forming three lips, each lip containing a small protuberance. On the surface of the head six punctiform papillae are visible; the dorsal and ventral ones are placed further to the front, and project more than the submedian ones, which are placed in shallow grooves.

The oesophagus is filariform, measuring $480\text{--}500\mu$ in length. Its anterior portion is long and slender, backwards it increases in thickness and is constricted somewhat suddenly before it goes over into the globular bulbus. This portion has a diameter of 48μ . The constricted portion about half ($20\text{--}24\mu$); in cross-section it is circular, its lumen is triradiated, dividing the oesophagus into three longitudinal portions, each portion containing muscles and glands (oesophageal glands).

Behind the bulbus are situated the intestinal valves, consisting of four cells, two of which seem to be fitted into the base of the bulbus and two lying outside. They join the bulbus up with the chyle intestine. This is formed by two rows of cells, a dorsal and a ventral one, surrounding the intestinal lumen. The individual cells are not recognizable; the nuclei, however, are recognizable, although not always distinctly. The rectum is more or less straight, proceeding in an oblique direction dorso-ventrally. The lumen is lined with a chitinous substance. At the end of the chyle intestine, where the rectum joins, three cells are visible belonging to the ligamentum intestino-rectale. Two are placed laterally and somewhat in front, and one dorsally and somewhat backwards. They are particularly distinct in the female.

The opening of the excretory pore is about 300μ behind the mouth opening. From this pore to the left and to the right a canal branches off, the branches gradually widening out in their course and joining up with the lateral band, in which on cross-section a fine lumen is visible.

Excretory System.

The two cervical glands abut in front of the branches of the excretory canal (bridge). The glands extend backwards ventrally from the bulbus and the chyle intestine a good distance into the body cavity. They are spindle shaped, tapering out in their posterior end. Each gland possesses a fairly large nucleus, placed one in front of the other. The contents of the glands are granular. Their posterior ends are sometimes doubled back, or cross each other above the chyle intestine.

Male Sexual Organs.

The male possesses a simple, viz., undivided bursa. (Fig. 8.) The rays originate with three roots. The dorsal ray is forked as well as the inner branch of the fork, thus six branches are found, three on each side. The bifurcation of the corresponding branches is not always equi-distant, the branches accordingly being of unequal length. The externo-dorsal ray is short and only reaches a little more than half-way to the margin of the bursa. The median root is unevenly cleaved, the base is fairly large; the postero-lateral ray is thin, bends dorsally and tapers out into the bursa without reaching the margin; the medio-lateral and the externo-lateral rays are of about equal thickness and run parallel to one another and are directed ventrally.

The two rays of the ventral root are of unequal thickness; the ventro-ventral ray is thin and ventrally bent; the latero-ventral ray is as thick as the two neighbouring ones at the median root, and runs parallel to them. It ends in the margin of the bursa. All rays end in a very fine papilla, which is most distinct in the externo-dorsal ray.

The prebursal papillae are ventrally situated and stand out like a thin thorn. They are easily overlooked. There are two spicules reaching a length of 140–158 μ . The anterior end shows a knob-like protuberance on to which the retractor muscles are attached. The spicule is longitudinally grooved, and along the groove is found on one side a convexly curved ridge. The caudal end tapers out into a spine, and there is a second spine, shorter and finer, originating dorsally from the main spine, in the latter third of the spicule. The anterior end of the spicule is hollow, and in front of the protuberance are two apparently empty spaces into which the spicule can be retracted. Behind the two spicules is placed the gubernaculum, in a curved dorso-caudal direction. It is light brown in colour in contrast to the dark brown colour of the spicules. When flattened out, the gubernaculum is lancet shaped with a short blunt spine directed backwards. The internal genital organs consist of a simple tube in which the testes, the seminal vesicle, and the cement glands can be recognized. The anterior end reaches to the posterior end of the cervical gland or pushes even alongside and reaches the base of the oesophageal bulb. It shows, on cross-section, the arrangement of the spermatogonia radiating along a central rhachis. A little further backwards, detached spermatozoa are seen. They are short and rectangular, with a short tail. The seminal vesicle is formed by a cylindrical tube, set off on both ends by a distinct constriction. It may be found teeming with spermatozoa. The ductus ejaculatorius is partially surrounded by the cement gland. The picture which Looss gives for *Ankylostoma* has here its analogy. The end communicates with a chitinous channel, which is confluent with the rectum to form the cloaca. This opens ventrally within the bursal membrane, projecting in the form of a small cone (genital cone).

Surrounding the ductus ejaculatorius ventrally and arranged in oblique dorso-ventral direction are the fibres of the constrictor muscles.

Female Sexual Organs.

The vulva is situated about a distance of 800μ from the tip of the tail, and opens ventrally through a slit placed crossways; sometimes this slit stands at an angle to the longitudinal axis. Ringed chitinous folds, a higher one in front and a lower one behind the slit, are present running longitudinally in the middle line of the body. A short vagina leads into the ovijector. This organ is composed of a middle piece, the ovijector proper or the pars ejectrix, to which join at each end a pars haustrix, connecting the uterus of the corresponding side. The apparatus has a length of about 300μ . The pars ejectrix is strongly built, lined by a well-folded membrane. On cross-section of an empty ejectrix the lumen appears in the form of a cross with forked branches. When eggs are present they are tightly surrounded by this membrane. The folded membrane is encased by a homogeneous wall, apparently chitinous, forming the skeleton or support of this organ. It is surrounded by the musculature which is arranged in a spiral curve around the organ.

The connection of the pars haustrix with the uterus is effected by a thick ring of muscles arranged radially to the axis. The lumen is lined with the folded membrane continuous through the connecting orifice of the pars haustrix. One uterus is placed behind (the posterior), and one before the ovijector (the anterior). They are simple tubes lined with cells. The posterior uterus is the smaller one. Before its continuation in the oviduct the lumen of the uterus widens out and then almost suddenly becomes narrow, leading into the oviduct. The widened out portion of the uterus is lined with large cells, radially arranged and in an oblique outward direction. It is possible that the secretion of the chitinous egg-shell takes place here as the eggs pass by. The orifice of the oviduct also has a lining of large cells; they are probably the sphincter muscles. The posterior oviduct describes a loop and turns in an oral direction along the uterus; the anterior oviduct is straight, and pushes its blind end up to and even along the oesophagus. Occasionally in its course one or more loops are found, and sometimes it is found making half to one turn around the chyle intestine. Generally speaking the course is a straight one. The eggs are relatively large and not numerous. In the posterior portion an average of 15 eggs were counted, and in the anterior one 30 eggs.

Nervous System.

In the constriction of the oesophagus is situated the commissure, which seen sideways has an oblique direction from the dorsal to the ventral side. Around the oesophagus are placed groups of ganglion cells; ventrally is a small group, the ganglion cephalicum ventrale; behind the commissure and touching it on its posterior margin, bilaterally placed, is the large group of ganglion cephalicum laterale, and a short distance behind it again, a smaller one, ganglion cephalicum post-laterale. Dorsally and behind the commissure is the distinct ganglion cephalicum dorsale. The distribution of these ganglia is entirely in conformity as given by Looss in *Ankylostoma duodenale*. The distribution of the nerves was not looked into, but it is likely that they too conform in these respects to *Ankylostoma*. The arrangement of ganglion cells in the posterior end of the body is not quite clear: there are several groups present.

Classification of the Ostrich Wire-worm.

The ostrich wire-worm belongs to the Strongylidae, and to the sub-family Tricho-strongylinac, in which there are five parasitic genera, Haemonchus, Trichostrongylus, Ostertagia, Nematodirus, and Cooperia. It comes nearest to the description of the genus Trichostrongylus, although it differs in shape, being of equal thickness practically throughout the whole length. The other characters tally within a few variations. The question arises whether the deviation from the shape is sufficient to warrant a separation from the genus Trichostrongylus, and to form a new genus. To our mind the differences are not sufficient, and accordingly we classify the ostrich wire-worm as Trichostrongylus douglasi (Cobbold), synonym Strongylus douglasi.

The Evolutive Cycle of the Ostrich Wire-Worm.

The egg was examined in fresh droppings. The shape of the egg is not a true oval, as a cylindrical middle piece connects the two poles, which are rounded somewhat bluntly on one and sharper on the other end. Occasionally a ventral and a dorsal side can be distinguished, considering the sharper curved one as the dorsal, and the flatter one as the ventral. In the optical cross-section the shell of the egg can be recognized as a well-defined line. Between shell and contents a transparent, apparently empty space is visible. The protoplasm has a granular appearance and is somewhat opaque. It is surrounded by a thin membrane, the vitelline membrane. It consists of a number of segment cells whose outlines are particularly distinct on the periphery. This arrangement is known as the morula stage. All freshly passed eggs are found in this stage. (Fig. 11.)

Size of the Egg.

Cobbold gave the length as 1/430th of an inch, i.e. 50μ , and its width as 1/750th of an inch, i.e. 30μ . We must keep in mind that Cobbold's material was preserved in alcohol, and was taken from the stomach of an ostrich. The measurements taken by us refer to eggs in droppings and not preserved. We registered as the most frequently found length 66μ , with a corresponding thickness of 41μ . This figure represents the culmen of the curve representing the variation in length. This curve drops more gradually when approaching the upper limits as compared to the lower end. The minimum was registered with 59μ , and the maximum with 74μ . The most frequent thickness was 39μ . A curve indicating the various thicknesses resembles the one described for the length. The minimum amounted to 36μ , the maximum to 44μ . There does not seem to be any regularity between thickness and length of an egg.

The Development of the Embryo.

Eggs in the unicellular stage are found in the uterus of the worm nearest the oviduct. In the ovjectors eggs are usually found undergoing division, showing most frequently two cells. The nucleus is not clearly visible; occasionally in lifting or descending the microscope tube a darker area is noticeable, corresponding about to the size of the nucleus. It is hidden in the centre of the protoplasm. Freshly deposited faeces contain eggs in an advanced morula stage, of which we can distinguish one with

larger and one with smaller cells. Their number cannot exactly be determined; in the large cellular morula not less than twenty are present. As the number of cells increases the empty space surrounding the contents becomes narrower, but never disappears completely. The small cellular morula shows on one side an indentation, seen sideways as an oblique cleavage entering from the margin; in different eggs it goes to a different depth. In a stage a little further advanced two portions can be distinguished, an anterior thicker one and a posterior thinner one; the thicker one tapering out into the thinner one, then doubling back and running alongside the anterior thicker portion. This is the head end. This stage is known as the tadpole stage. Subsequently a longitudinal growth becomes marked more and more, and the typical shape of a worm is formed. The larva is finally found coiled up. As already mentioned in the ovjector the eggs are found in the two-cell stage; in the droppings they are found in the morula stage. Then whilst they pass through the intestines of the bird, the development continues. The time elapsing between the depositing of the eggs in the stomach and their appearance in the droppings is about three to four days. This fact was ascertained by drenching a clean bird with eggs suspended in water. The hatching of the egg is preceded by active movements of the larvae.

The Larva.

Maupas, as a result of his investigations into free living nematodes, drew attention to the fact that four ecdyses are observed. He drew the conclusions that these will prove to be the rule for all nematodes, and in support of his view referred to the findings of Looss, who likewise in the cycle of *Ankylostoma duodenale* described four ecdyses. The rule, therefore, is probably also correct for parasitic nematodes. We may state at once that we were able to find four ecdyses in our parasite, corresponding to four different stages. Two of these stages are met with in the cycle outside the host, and two inside the host. The cycle can thus be divided into a parasitic and a non-parasitic period. Each stage can be sub-divided again into three phases, viz., one of growth, one of lethargy, in which the structural changes take place, to be followed by one of activity, which finishes with the casting of the skin.

The Larva in the First Stage.

The description is taken from older larvae. In form it resembles an imago; it varies in length (according to whether freshly hatched individuals or older ones are measured) from 240–750 μ , and in thickness from 18–28 μ . The body tapers from its thickest diameter directly behind the oesophageal bulb to the oral and caudal end. The tail gradually tapers out towards and continues into a thin cylindrical appendix, carrying at its extreme end a very small knob. The appendix can only be recognized under a very high power (immersion). *It is typical for the first larval stage.* (Fig. 1.) The mouth end has the narrowest circumference of the head. Its margin is bent inwards and widens off, opening into a funnel-shaped vestibule which continues in the straight oral cavity.

Placed around the mouth opening are six punctiform papillae. Behind the mouth margin, and parallel with it runs a very superficial constriction. The head seems to be of a rigid structure, and the mouth aperture to be constantly open. The walls of the mouth cavity appear in the optical cross-section as two well-defined lines; on both ends, but in particular on the caudal one, are small punctiform knobs. They are optical cross-sections of a ring circling around the two ends of the mouth cavity. The posterior ring is the thicker of the two.

The oesophagus is rhabditoid in form. There are three portions, an anterior club-shaped one succeeded by a fairly narrow constriction leading to the globular bulb. In its interior is placed the so-called Y valvular apparatus, which is frequently found in activity. The first two cells of the intestinal canal are smaller and appear somewhat compressed. They present the primordium of the future valvular arrangement between the oesophagus and intestines. The lumen of the intestine, observed from the lateral side, has a zigzag course and is lined with fairly large cells. The rectum has a dorso-ventral direction and is straight. The cuticle surrounding the anus is slightly raised.

About in the middle of the body and below the intestinal canal and in an angle to the longitudinal axis is placed the sexual primordium. It is oval in shape. The chitinous cuticle of the larva is first quite smooth, later it is finely ringed. The intestinal cells are filled with numerous fine granules, these rendering the small creature opaque. It seems that this opaqueness increases with the growth of the first larval stage. Growth is the principal character of this stage. It is followed by the first ecdysis. A new cuticle is formed. Subsequently along the contour of the larva a double line appears, and when the worm is in activity, wrinkles in the convexity of the curves. Later the head separates from its cover and withdraws within and also the tail end. Finally the larva is found loose in the skin. The formation of the new skin takes place whilst the larva is in a state of lethargy. It is active again as soon as the structural changes have taken place. The first stage has then reached its end, and the young larva inside the old skin is at the beginning of the second stage. It has a blunt tail end, which is characteristic of the second stage. The first skin is only retained for a very short time; indeed this stage is quite of a passing nature, provided the outside conditions for further growth are favourable. The casting of the skin begins by the detachment of the oral end in the form of a hood, which frequently remains attached to one side of the skin, like a cover on a cylindrical box. The larva leaves the skin under great activity, moving the body in all directions. In the cast off skin the cuticle of the punctiform papillae, the lining of the mouth opening, and the lining of the anus are present. The skin is sometimes broken in the process of moulting. Under the most favourable conditions the first moulting process is completed fifty hours after the evacuation of the faeces.

The Larva in the Second Stage. (Fig. 12.)

The growth begins immediately after the moulting, and also takes place very rapidly. It leads to the formation of the second ecdysis. The description applies to a larva in the mature stage. The opacity of the

intestinal cells still present at the beginning of the second stage and due to the presence of fine granulations of different sizes, disappears in older larvae which are more or less transparent. The details of the internal structure now show up quite distinctly.

The initial length of this stage averages 600μ ; when concluded 900μ . The body is of a more slender form than previously. There are no changes at the head. The tail is drawn out somewhat longer and finer, and its end is rounded off. *This is characteristic for the second stage.* (Fig. 2.) The lateral bands are now distinct. They taper out on both ends.

The presence of fine rings are noted in the cuticle, deeper in the middle than in both ends of the body. The lateral band is placed below the cuticle, is well defined and possesses at intervals a number of nuclei, usually arranged in pairs, behind or opposite to each other. The body muscles can be recognized, but are ill defined; their nuclei, however, stand out very clearly. The ventral band is also fairly distinct, and possesses a great number of small nuclei following each other very closely. In the region of the oesophageal bulb and the anus, the nuclei occur in groups. According to Looss these groups are the primordia of the future cephalic and anal ganglia. The papillae around the mouth opening are slightly raised; two, the dorsal and the ventral one, stand somewhat in front of the other four, the submedian ones standing bilaterally to the main sagittal plain through the head. The mouth opening and cavity correspond to the description of the first stage. The oesophagus is still rhabditiform, increased in length, particularly in the anterior portion; in proportion to the whole body length the oesophagus, however, appears to be shorter than that of the first larval stage. The valvular apparatus is still present. The chyle intestine begins with the two cells mentioned before. The intestinal cells are arranged in rows. Each cell contains a large nucleus and bulges out in the direction of the lumen, which has a zigzag form. There are eight dorsal and eight ventral cells. The first cell is the shortest, about half of the ordinary length. The remainder are equal in thickness and vary in length from 13μ to 22μ . The thickness amounted to 4μ . The rectum has a dorso-ventral direction, the anus opens with a slit placed crossways, the posterior margin is slightly raised. Behind the anus are a group of cells; they correspond to the future pulvillus.

The porus excretorius and its canal are distinct and are ventrally placed below the oesophageal bulb. The commissura cephalica is clearly visible as a band surrounding the oesophagus in its constricted portion. Seen laterally it is directed obliquely in a dorso-ventral direction. Behind the commissure is a collection of distinct nuclei, arranged in two lateral and one median groups. The former two correspond to the ganglion cephalicum laterale. The nuclei are closely packed and reach backwards to the middle of the oesophagus and more loosely arranged even behind the bulbus (ganglion postlaterale cephalicum). Dorsally is placed the small ganglion dorsale. The ventral group behind the commissure reaches as far as the end of the bulbus; it corresponds probably to the primordium of the excretory apparatus. Behind it is the ganglion cephalicum ventrale, already alluded to in the description of the ventral

band. In the anal region on each side is found a small group corresponding to ganglion lumbale, and ventrally the ganglion anale, also already mentioned. Laterally to the commissure and ventrally are also groups of nuclei arranged in a forward direction. Particularly distinct are a number of rows of long individual cells gradually decreasing in size in the direction of the head, the space between the two cells increasing as well. These cells probably correspond to the future papillary nerves.

The sexual primordium is placed in the middle of the larva; in the first larval stage it consists of two cells, in the second stage it appears larger and composed of a number of cells.

The separation of the old skin is a prominent feature, a double contour being clearly recognized. This occurs during the period in which the larva is found in lethargy and is followed by a great activity of the now mature larva. After a minimum period of sixty hours, under most favourable conditions, maturity is reached, and the new larva inside the old skin is found detached and an empty space is found in front and behind it, the natural openings being closed. The tail end is again quite typical of the enclosed larva, and will be described under the third stage. The rings of the old skin are closer than those of the new one found underneath. There are about two rings of the old skin to one of the new skin. There is no difference in the casting process of the skin from that described before. The casting was, of course, not observed under the natural conditions under which it takes place. It could be demonstrated by means of a dilute acid, under the influence of which a great number cast their skins. Considering that stomach juice is probably acting as a stimulus for the casting of the skin, larvae were placed in such an artificial digestive juice (a mixture of pepton and hydrochloric acid) and the casting of the skin took place readily. It was found, however, that diluted hydrochloric acid alone is sufficient, and finally the same observation was made with any other acid used (nitric, sulphuric, acetic). It appears that the dissociated hydrogen ion is responsible for the stimulus.

The Larva in its Parasitic Stage.

The Third Larval Stage.

It may be called the transitory stage to the sexual one. A typical feature of this stage and one of diagnostic value is a small knob on the point of the tail having a prickly appearance and resembling somewhat the fruit capsule of *Datura*. (Fig. 3.) This prickly knob is naturally already seen within the old skin of the mature larva. Larvae collected from the stomach of an ostrich three days after it had been drenched with larvae suspended in water are found with this prickly tail end; the larva after casting the skin is shorter than before it has stripped the skin. On the 3rd day already it has outgrown its former length. The measurements taken were as follows:—

Mature larvae with the old skin averaged 745μ ; without the skin 530μ . On the 3rd day the length reached was 815μ ; the thickness was 24μ . The main internal change in this stage affects the sexual primordium. From its former oblique position, it goes

over in a longitudinal position. The number of cells is increased and arranged into an oval, the central cells are larger; those on both poles are smaller. The length of this organ on the 3rd day varies between 8-32 μ . With but few exceptions the shorter primordia are nearer the middle of the body, the longer ones are towards the end, the longer they are the nearer to the end. This feature represents already a sexual differentiation, the larger primordia reaching further backwards are the future females. This is particularly clear on larvae six days old. The distance between anus and tail varies but little and averages about 80 μ . During this third stage the rhabditiform oesophagus changes into a filariform one. The so-called valvular or tooth apparatus within the bulb disappears. A new skin is not formed within the first three days, but in larvae removed six days after drenching a double skin is noticeable; in a number of larvae a double contour of the cuticle lining the rectum is clearly defined. The new larva within is again shorter as before; there is an empty space in the old skin before and behind. The connection between old and new skin seems to be particularly persistent in the region of the anus, due to the rectum, whose cuticular lining does not seem to detach so easily.

On the 6th day the majority of the larvae have moulted. The sexual primordium of the female larva has stretched itself considerably, and is placed towards the posterior end. It is now spindle shaped; its central cells are large, the end cells are small; it measures about 40 μ in length. This arrangement, in particular the large cells in the middle, are typical for the female. Occasionally there is an indication of the future vulva in the shape of slits placed crossways in the ventrally placed middle cells.

In larvae three days in the host no double contour of the body was noted, but on the 6th day the great majority had cast their skins. The ecdysis and the casting of the skin accordingly would take place within these two dates. A control experiment was carried out to fix this date more accurately. For this purpose an ostrich was drenched with mature larvae suspended in water and was killed 4 $\frac{2}{3}$ days later. Now about half of the worms examined had already cast their skins and the other half were in possession of both old and new ones; they were found detached within the old one. Thus 4 $\frac{2}{3}$ days represents the average time required for the development of the third stage. The skin with the prickly tail end is the cast one.

The Larva in the Fourth Stage.

It may be called the stage of the sexual differentiation. Its average duration is probably fifteen days, inasmuch as on the 21st day after drenching the majority of larvae have already moulted, or are in the process of casting the skin. It may last up to the 27th day, when rare numbers are still encountered with the double skin. After the casting of the outer skin, the new larva again is somewhat shorter than previously. But since the growth takes place very rapidly, the moulted larvae found on the 6th day have already outgrown the not yet moulted larvae of the 3rd day. The length on this day is about 1000 μ by 30 μ thickness as compared to 815 μ by 24 μ on the 3rd day.

On the 9th day the length has doubled, its average being 2140μ and the thickness 44μ . The two limits on this day are a minimum of 1600μ and a maximum of 2480μ respectively. The following figures give the growth during the succeeding period.

				LENGTH.			THICKNESS.		
				Minimum.	Maximum.	Medium.	Minimum.	Maximum.	Medium.
12th Day	1880^{μ}	3200^{μ}	2466^{μ}	36^{μ}	59^{μ}	46^{μ}
15th Day	2820	3520	3132	50	60	53
18th Day	Male...	...		3140	3720	3104	32	58	57
	Female	...		2560	3440		54	62	

The tail has again undergone a change, now indicative of the sex. (Fig. 4.) It is shorter than previously. The average length from the anus to the tail end is 56μ , in the third stage this distance was 80μ . Also the shape has changed. The dorsal line runs straight with the back.

The cuticle is ringed. The lateral band is distinct and about 10μ in width; the nuclei therein are flat and oval. Each nucleus contains one or more nucleoli. The ventral band is also distinct, and its nuclei are closely packed. The muscles are rhomboid shaped long drawn-out cells, four in a quadrant just behind the head with longitudinal striations. The nucleus is placed on the inner protoplasmic portion of the cell.

The mouth opening is distinct and continues funnel shaped inside, so that on optical cross-section an opening with two opposite lips is simulated.

The oesophagus is outspoken filariform, its anterior portion slender. The so-called tooth apparatus in the bulbus has disappeared. The two cervical glands are particularly clear in this stage; they are ventrally under the oesophagus and abut orally on the so-called bridge, which is formed by the canalis excretorius branching off laterally towards the two lateral bands. They possess a large nucleus, oval shaped, placed behind the oesophagus and one behind the other. The gland has granular contents. At the beginning of this stage this gland is short, it grows out backwards and pushes along the chyle intestine. Occasionally the gland shows one or more kinks and coils around the intestinal canal. The posterior end tapers slightly and is rounded off. The chyle intestine shows no peculiarity. There is an increase of intestinal cells; they possess the same form as before. At the beginning of the intestine are placed the four cells forming the valvular apparatus. They do not appear to have undergone any change yet. The cells belonging to the ligamentum intestino-rectale are particularly striking by their size.

In the succeeding days males and females can be recognized by the tail. It is shorter in the male. Measurements taken on the 6th day show the longest distance from anus to tip of tail to be 60μ in the male, whereas

the shortest in the female was 64μ and the longest 80μ . They also differ in form; the ventral side of the male is curved with the convexity directed dorsally; the curvature begins at the anus, in the female this ventral line is more or less straight. On the 6th day the primordium is differentiated. The male primordium lies nearer the middle of the body. The distance from anus to end of primordium in the male measures from 120 – 180μ , as compared from 64 – 84μ in the female. Correspondingly varying are also the lengths of the primordium; that of the male is shorter. The longest male primordium measured 44μ , the average length was 12 – 24μ ; in the female it varied between 100 and 114μ . The male primordium is oval shaped with a double row of cells; the oval stretches itself backwards, i.e. it grows backwards. It grows also in the anterior direction, so that three different portions can be distinguished, a middle piece and two ends. The middle piece consists of two rows of cells; the end piece of one row, the ends are somewhat club shaped. The anterior piece is longer than the posterior. Measurements taken in larvae of the 9th day were: middle piece 80 – 124μ , anterior end 200 – 240μ , posterior end 92 – 100μ . The posterior end at this time does not yet reach the region of the recto-intestinal ligament; only occasionally the end pushes as far as the large cells of this ligament, under which it passes to join later the cloaca. The club-shaped end continues into a small appendix, visible as long as it has not joined up with the cloaca. During the following twelve days this anterior cell string, hitherto solid, begins to show a lumen as well as the middle piece. It appears as a duct whose walls are lined with cells, the anterior end being still a solid cell string. The middle piece is probably the forerunner of the future seminal vesicle. On the 15th day very few changes have taken place; the middle piece is hollow and empty; the posterior end shows a double row of cells; the anterior end is solid. On the 18th day the moulting may be completed. Further changes take place in the imago stage, to be described later. During this time the formation of the bursa takes place. The incipient stages can be detected as early as the 6th day. The chitinous rectum detaches itself dorsally from its surroundings, along its course a cleft is formed, which gradually extends around the rectum and extends forwards and backwards. It is the forerunner of the future cloaca. The rectum is finally hanging free in this empty space, attached on one side to the anus and on the other to the chyle intestine. On the 12th and 15th days the primordia of the future spicular apparatus and the bursal rays are distinct; in particular the former is in the shape of a large cell with a protoplasmic appendage running backwards. On the 15th day some larvae already possess a spicule, with the gubernaculum and the bursa fully developed. On the 18th day most of the larvae possess the double skin; the spicules and gubernaculum have formed, the rays of the bursa rolled inwards and enveloped in the detached old skin. The spicules are transparent, averaging 158μ in length; the pocket at their anterior end is present. The cloaca is distinct; the sharp points of the spicule are pushed into it through a narrow slit.

The female primordium can in many cases be recognized as such on the 6th day in the moulted and not moulted larvae. It is spindle shaped, with larger cells in the middle and smaller ones on both ends, arranged in a double row. The central cell is the biggest of the lot; ventrally it soon

splits crossways to the longitudinal axis, indicating the future vulva. This cell is also raised slightly above the adjoining ones and reaches the subcuticle. In a somewhat more advanced stage this slit is surrounded by a number of cells. The changes noticed on the 9th day affect in the first instance the size of the organ. The primordium has grown and measures from 216-360 μ . The two ends are slightly club shaped. On the 12th day the middle piece is found to be hollow. The future form of the ovijector and uterus is present in its outlines, in the shape of a sand-glass, in some of the more advanced individuals. It is lined with large cells, the lumen is narrow. On the 15th day the vulva is distinctly raised, it leads into the vagina about in the centre of the middle piece of the ovijector. At the base of the vagina two cells are particularly distinct, they seem to form a ligament connecting it with the ovijector, standing out ear-like. The vulvular slit is surrounded by four large cells, two in front and two behind. Where these two cells join sideways a third one is superimposed, not reaching the slit. The ovijector is hollow, and the future pars haustrix separated off. On the 18th day the larva in its new skin shows the ovijector in its final form lined with the folded membrane; the pars haustrix forms a distended vesicle. The empty uterus is lined with long cells radially arranged; its lumen is distended where it joins to the oviduct. The two horns of the uterus are long and sometimes form loops. The vulva is raised, the vaginal portion is pear shaped; at its base the already mentioned cells are still very prominent. The lumen of the vagina beginning at the vulva is straight; before its entrance it forms a spiral curve. It is lined with chitin.

It has already been stated that the majority of larvae moult between the 18th and 21st days. On the first-mentioned date nearly all the larvae possess the double skin, and on the 21st day, the great majority have stripped them. On this date a number are yet found in the process of moulting, i.e. with partially stripped off skins. There are no peculiarities in this last moulting process. On the 24th and 27th days still a few larvae are found in the old skin. (Figs. 5 and 6.)

The Larva in the Fifth or Imago Stage.

After the fourth moulting the final form of the worm is reached, and further developments lead to final maturity. The newly hatched larva is again shorter than before. The growth is very rapid. The following averages were found:—

	Male.	Female.
21st day.....	3470 \times 67 μ	3660 \times 67 μ
24th day.....	3210 \times 65 μ	4110 \times 66 μ
27th day.....	3540 \times 60 μ	4470 \times 67 μ
30th day.....	3770 \times 66 μ	4280 \times 69 μ
33rd day.....	4250 \times 74 μ	5390 \times 79 μ

There is a noticeable difference between male and female; the former is as a rule shorter, although they are sometimes of equal length.

The bursa is in all details complete. The three roots of rays are distinct; the ventro-ventral is split off the latero-ventral one about in the

first sixth of its length. At the place of bifurcation the width of the root is 22μ ; the ventro-ventral ray at this place is 6μ broad, it bends ventrally and finishes in the margin of the bursa.

Latero-ventral ray, externo and medio-lateral run parallel. At the base the diameter measures 44μ ; the individual rays have about equal diameters; their tips are in the margin of the bursa; the ends of the first two bend ventrally, that of the last dorsally.

The postero-lateral ray at its widest diameter measures 9μ ; the externo-dorsal ray does not reach the margin; its broadest width measures 10μ . The ray gets wider as it approaches the root, whilst the lateral rays are narrower, so that the lateral root measures only 20μ . The medio-dorsal ray is forked, and the branches again are forked. The surface of the rays are pennately striated. The length of the spicule is 158μ , that of the gubernaculum 64μ .

The testes show the spermatogonia in a pennate arrangement around a common axis. The vesiculum seminalis is empty, well defined on both ends. The posterior portion of the seminal duct is fairly broad and filled with cells (cement gland); the end piece is lined with chitin.

On the 27th day the nucleus of the spicular primordium is still present as part of the spicule itself.

On the 23rd day the seminal vesicle is found filled with spermatozoa; it is very broad, even broader than the chyle intestine. The papillae situated before the bursa are distinct.

In the female the vulva is raised, the cuticle in the middle sagittal line is raised to a fold, forming a ridge separated in two by the vulva. The ovijector shows a kink pushing the vulva outwards. The ovijector and adjoining uterus are complete. The ovaries have not yet reached their full length; they do so about the 30th and 33rd days. The anterior ovarian tube pushes itself between the cervical glands; the posterior tube is bent forward at its junction to the uterus and its end reaches about the middle of the body.

Eggs are present on the 33rd day; at this date they are already present in the ovijector.

Bionomics of Trichostrongylus douglasi.

The non-parasitic stages of the ostrich wire-worm are free-living and from their entrance into the body of an ostrich depends entirely the future chance of development and propagation. It is, therefore, conceivable that they will be endowed with such qualities as will secure that end. The knowledge of these qualities has a practical importance inasmuch as it will lead to deduction how the wire-worm plague can be attacked outside the ostrich.

It refers principally to the infectivity of the various stages, their longevity, influence of environments, and habitat.

(1) Infectivity of the Various Stages.

The first stage is the egg. It leaves the stomach with the faeces. The number present in the faeces varies very much in different animals and indicates the degree of infection in a bird. The number of eggs in a given quantity of faeces counted and calculated for the total faeces passed in a day, can be given in a more or less accurate degree. The

droppings of an ostrich were examined during a period of ten days and the eggs present in one gramme were counted, and the following numbers were found:—1400, 2400, 2000, 3200, 4000, 2400, 1600, 1200, 800, and 1200, or an average of 2000 eggs per gramme. In order to obtain an idea as to the average amount of faeces passed per day, the droppings of two birds, confined in the same cage, were collected during the same period, and an average of $1\frac{3}{4}$ kilo, per bird and per day, was registered. Taking the average daily dropping to be 1750 grammes, and the number of eggs per gramme to be 2000, then the ostrich in question passed daily an average of 3,500,000 (three and a half million of worm eggs). To judge from the appearance of the bird, which was in good condition, being well fed, one was not in the position to state whether this figure represents a gross infection. The condition of the bird would speak against such an interpretation. The following figures represent the number of eggs per gramme of faeces collected from ten different birds, weight of excretion in grammes which had passed when collected; the number of eggs calculated per excretion:—

Eggs per gramme.	Weight of Dropping.	Eggs per Dropping.
200	120 grammes	24,000
400	110 "	44,000
800	70 "	56,000
1,600	25 "	40,000
1,600	80 "	128,000
1,600	90 "	144,000
2,000	40 "	80,000
2,000	90 "	180,000
2,400	125 "	300,000
12,000	50 "	600,000

From these figures it can be seen to what extent the faeces of a bird can be infected. With the exception of one bird, the one with the greatest number of worm eggs per unit weight, all were in excellent health. It appears thus that, provided there is proper and sufficient food to keep the bird in good condition, the wire-worm infection is not felt so much by the bird. This observation would bear out what ostrich farmers know, that wire-worms are no trouble provided both birds and worms are fed well. The figures given are also an indication of the rapidity with which the infection of an enclosure can take place. The conclusions may safely be drawn, that the rate of infection of an enclosure stands in direct proportion to the number of birds present, and the danger of infecting birds is the greater the smaller the enclosure in which they are kept. This being so, the question naturally arises, how long does a bird remain infected? *A priori* it is evident that it must remain infected as long as it is kept on an infected farm. The object, however, is to know for how long a bird drops wire-worm eggs when prevented from reinfesting itself; in other words, for how long a period does a bird act as a "reservoir of virus," to use an expression which adequately describes the position. This period as observed in one instance is at least nine months, during which time the infection in no way abated. Judging from analogy with *Ankylostoma* in man, it probably will last for years.

(2) *The Hatching of the Egg.*

As already stated, eggs in fresh droppings are found in a multicellular stage, but none have been observed yet containing a fully developed embryo ready to hatch. It was of some interest to know whether eggs, as such when ingested, would develop in a bird. Before the life-history was fully understood, this question was settled more to meet a practical than a theoretical issue. Ostrich farmers give newly hatched birds droppings from an old ostrich, and they assure us that under natural conditions a young bird picks droppings first, and accordingly many farmers believe that fresh droppings from the mother bird are necessary for a young bird and supply it to the chicks hatched in the incubator. It was also thought that with such a practice worm infection could be transmitted. For this purpose two ostriches were fed daily with droppings about $\frac{1}{2}$ –3 hours old during a period of six weeks. The number of eggs fed were calculated and found to amount to five million in the case of the one, and ten million in the case of the second bird. Every day the droppings of the ostriches were collected and placed in jars. An examination of the droppings showed that they contained eggs, and these eggs hatched in the jars. They had appeared for the first time four days after ingestion, and were present as long as the birds were fed with fresh manure. On discontinuing feeding with ova, the droppings ceased to be infective, proving the absence of adult worms in the ostriches. After the lapse of eighty days one of the birds was killed, and on examination of the stomach no worms were found. It appears thus that worm-eggs cannot infect an ostrich; they pass the intestines unhurt; they require about four days for this journey and continue their development afterwards.

It is apparent that the worm-egg hatches outside the body of the ostrich. It is evident that such hatching only takes place under favourable conditions which must have their optimum and will be confined within certain limits.

A number of questions were put and answered by the experiments:—

(1) *The Optimal Temperature for Ova to Hatch and Develop into Mature Larvae when kept in Fresh Droppings.*

From an experimental point of view the question put in this way could easily be controlled by a culture, made with the faeces of an infected bird, the appearance of larvae on the walls of the glass jar in which the droppings were placed allowing an easy examination. The results were as follows: At a temperature of 81–82° F., ova hatch and larvae mature in ninety-eight hours; at a temperature of 90–91° F., sixty-seven hours were necessary. At a temperature of 95° F. after sixty hours mature larvae were observed, and at 98° F. in fifty-nine hours. It appears thus, that with an increasing temperature to 98° F. in moist droppings and asmosphere, the rate of development is accelerated. This development embraces hatching, first and second ecdysis.

(2) *Hatching of Ova and Development of Larvae below the Optimal Temperature.*

Fresh droppings were placed in the ice chest on 5/6/12 and kept until 16/7/12. The temperature observed varied between a minimum and a maximum of 36–40° F. During this period the droppings were examined

five times and no larvae were found. At the conclusion of the experiment (16/7/12) the droppings were brought to a temperature of 93° F. to allow of hatching. Only a few larvae developed and reached maturity.

In a second experiment extending over the same period the temperature oscillated between 38° F. and 44° F. The results were identical. It appears thus that at a temperature of 44° F. and below, the hatching of eggs does not take place at all, or at least all development is retarded.

In the third experiment, commenced on 23/7/12, fresh droppings containing fairly numerous ova were placed in the ice chest until 15/9/12, and the following observations were registered:—

1/8/12. (Minimum temperature 44° F., maximum 45° F., between 23/7/12 and 1/8/12.) A few of the eggs were found to contain embryos.

6/8/12. (Minimum temperature 44° F., maximum 46° F., between 1/8/12 and 6/8/12.) The majority of the eggs were found to contain embryos; no larvae were noticed.

12/8/12. (Minimum temperature 43° F. and maximum 46° F., between the 6/8/12 and 12/8/12.) The embryos in the eggs were noticed to move within the shell, and for the first time free larvae were noticed. Their length measured 320 μ (first stage).

17/8/12. (Minimum temperature 44° F., maximum 46° F. in the preceding interval.) Eggs with moving embryos, free living larvae (first stage), and *some dead larvae were found*.

23/8/12. (Temperature 45° F. to 48° F. in the preceding interval.) The larvae now reached a length of 300–400 μ (first stage). The majority of the larger larvae are found dead.

28/8/12. (Temperature 45–48° F. in the preceding interval.) The larvae now reach a length of 360–600 μ . The majority of the small larvae are found dead.

3/9/12. (Temperature 44° F. to 50° F. in the preceding interval.) The length of the larvae varies from 360–600 μ . There were only two of the latter length.

9/9/12. (Temperature 43° F. to 47° F.) The living larvae measured 561 to 800 μ , all smaller larvae were dead; the largest dead larvae found measured 560 μ .

15/9/12. (Temperature 43° F. to 48° F.) Only a few larvae were found to reach 800–900 μ , none had reached the mature stage (second ecdysis). At this date the culture was placed at a temperature of 94° F., and it was noticed that only a few larvae reached maturity.

From the foregoing notes it appears that at a temperature of 44° F. to 46° F. the ova hatch but that the larvae die off. When the temperature is slightly higher (23rd and 28th of August) the larvae are able to grow as indicated by their length (400–600 μ). When the temperature dropped again to 43° F. (30th September), smaller, younger live larvae were no longer found; the larger ones, however, survived. It can therefore be concluded that at a temperature of 43° F. to 48° F. ova hatch, but the larvae do not reach maturity.

In a fourth experiment (*vide* Appendix I), extending over the period 23/7/12 to 15/9/12, during which time the temperature varied between 45° F. to 50° F., the majority of the larvae reached the mature stage (second ecdysis).

(3) *Hatching of Ova and Development of Larvae above the Optimal Temperature.*

Droppings of two birds showing numerous worm-eggs were collected during a period of ten days and kept at room temperature. They were then all placed in an incubator fixed at 98° F. These droppings thus contained eggs and larvae in all stages when examined eight days later. At this temperature the hatching of the eggs and the further development of the larvae was not interfered with.

Droppings of the same two birds kept in a similar manner as above were placed at a temperature of 100–104° F. for a period of eight days, and they were then withdrawn and kept at room temperature for a further ten days to allow the development of the surviving worms. It was found that under these conditions the larvae of the first collected droppings were in no way affected and reached maturity. Droppings collected within the last three days, however, and placed in the incubator, no longer showed worm cultures, only a few worms survived, whereas the control cultures kept at room temperature showed numerous mature larvae.

In droppings collected and treated as above and placed at a temperature of 102–102.5° F. for seven days, and then at room temperature for two days, no more larvae developed in the droppings that had been collected within the last three days before placing them in the incubator; in droppings collected within the four days only a few developed, whereas in the older droppings in which the larvae had reached the mature stage before they were placed in the incubator kept alive. The controls kept at room temperature showed numerous larvae.

Droppings collected over a period of seven days were simultaneously placed in an incubator at 104° F. and kept there for five days. After a stay of two days at this temperature no larvae were found in the droppings collected within the last few days, whereas in the older ones the larvae were found alive. After a sojourn of five days at a temperature of 104° F., however, all larvae were dead.

The results of these observations may be summarized as follows:

At a temperature of 98° F. ova hatched and larvae reached maturity.

At a temperature of 100 to 101° F. ova hatch, but only older larvae reach maturity.

At a temperature of 102–102.5° F. ova hatch, but only older larvae reach maturity.

A temperature of 104° F. does not permit the development of the larvae; even mature larvae perish.

The Influence of Moisture and Dryness on the Development of Ova and Larvae.

Droppings were collected on 12/2/12, and they were dried at room temperature in open dishes. They were utilized for test cultures to ascertain whether the larvae so treated would develop, and if so, for how long a period.

1. 19/2/12. *First test.* The cultures showed frequent larvae.
- 20/3/12. *Second test.* Larvae more frequent.
- 4/4/12. *Third test.* Larvae fairly frequent.
- 7/5/12. *Fourth test.* Larvae present.
- 10/6/12. *Fifth test.* Larvae present.
- 10/8/12. *Sixth test.* Larvae fairly frequent.

On this date a microscopical examination of the caked droppings, after moistening them, was made, and eggs with living embryos were seen; one larva was observed in its moulting process (first ecdysis); one living larva measuring 500μ was found in its (first) skin. There were present living larvae measuring in length from 240 to 720μ .

12/11/12. *Seventh test.* Some caked droppings were moistened. One or two hours later live larvae were fairly frequent and of different lengths (240, 260, 280, 320, 360, 400, and 420μ), representing larvae of the first stage.

29/1/13. *Eighth test.* A morsel of the droppings was moistened and immediately placed under microscope. Individual eggs or larvae were then focussed and kept so long under observation until it could be definitely stated whether they were dead or alive. The following notes were made:—

(a) One egg with an embryo; the embryo showed movements within two hours. (b) One egg with an embryo; the embryo revived and hatched within one hour. The larvae measured 240μ in length. (c) One larva measuring 240μ revived. (d) One larva measuring 280μ revived. (e) One larva measuring 360μ revived. (f) Three larvae measuring 400μ revived. (g) One larva measuring 440μ revived. (h) One larva measuring 560μ revived. (i) One larva measuring 600μ revived. (k) A larva measuring 480μ , and another 560μ , had their outer skin (first ecdysis) formed. (l) There were also larvae present up to 720μ in length which did not survive. The drying of the dropping at room temperature was not a sudden process. Moisture was gradually lost, and it was, therefore, possible for a number of eggs to hatch and for larvae to reach the first ecdysis. Naturally all intermediate stages had also to be expected, but what was expected did not happen, i.e. the survival of eggs and mature larvae only. As results proved, any larvae in the first stage arrested by desiccation could move after *having been dormant in a dry state for practically a year.*

19/5/12. *Ninth test.* Dry faeces kept for fifteen months were moistened and kept under observation. Three days later mature larvae were found on the walls of the culture glass.

12/7/13. *Tenth test.* The seventeen-month-old dry droppings were moistened and placed at a temperature of $90-92^{\circ}$. After two days a few, and after four days fairly numerous mature larvae were found on the wall of the culture tube.

12/2/14. *Eleventh test.* The remainder of the droppings were moistened, and when examined a week later a few mature larvae were observed on the wall of the culture tube.

The results can be summed up as follows:—Ova and larvae of the first stage, kept dry in the open at room temperature for two years, developed to maturity when moistened.

The Influence of Complete Dryness on Ova and Larvae. (Appendix II.)

Droppings freshly collected were placed at room temperature in a vessel containing calcium-chloride. The weight of the moist dropping was 18.6 grammes when the experiment started (5/3/12), when practically all moisture was absorbed it had lost 11.3 grammes in weight. Small parts of these dry droppings were, after certain intervals, moistened in order to see whether larvae would develop. Observations were continued for six months, and all attempts at culture proved successful, viz., the larvae developed.

This experiment was repeated with droppings desiccated above calcium-chloride at a temperature of 50° to 60° F. Droppings were desiccated for twenty days at this temperature, and then at ordinary room temperature. The first observation after twenty days desiccation showed that larvae were present; three weeks later living larvae could still be found; after another three weeks no more larvae developed. It appears thus, that at this low temperature, where desiccation is retarded, eggs do hatch when desiccation has become complete and further development is arrested.

Another variation of the experiment was to desiccate droppings completely at 98° F. For this purpose droppings were rapidly dried in an incubator for ten days, and then for another ten days placed above calcium-chloride at 98° F., and afterwards kept at room temperature. When subsequently examined after three weeks, six weeks, and ten weeks, mature larvae were found in the test culture. Four months after desiccation had started some of the droppings after moistening were microscopically examined, and eggs with embryos were found which hatched within an hour. Six and eight months after commencement similar results were obtained.

On the 22/1/13, when the droppings were ten months old, the following results were obtained:—One hour after moistening the first moving larva was observed. The examination revealed larvae of 200–400 μ length only. After 24 hours at 93° F., the larva had attained a length up to 720 μ , and after 48 hours up to 850 μ , but no mature larvae could be found. After four days the mature larvae appeared fairly frequent on the tube wall.

Further examinations were made 12½ months, 15½ months, 24 months, and 30 months after the commencement of the experiment, and in every instance the larvae were still able to reach maturity. Only after 36 months were the tests negative.

The test made on the 26/3/14 (after two years) revealed, fifteen hours after commencement, live larvae of 240 to 320 μ length. When examined after a week, mature larvae were present in the test tube. To prove their vitality, the mature larvae were dried and kept for twelve months. When moistened, a few of the larvae revived at once, and were still alive after being kept a week in water. The results can be summarized as follows:—Ova and larvae in the first stage do not develop further when kept dry, yet keep their vitality in a state of dryness for a period of three years (in one instance for six months) above calcium chloride.

The Influence of Moisture on the Development of Ova and Larvae.

Under the conditions of dryness ova do not hatch, and larvae do not develop. The question of moisture was considered both relating to the

atmosphere and to the medium in which eggs were exposed. The first point was settled by placing dry droppings above water under a glass bell so that the atmosphere of the bell was saturated with moisture. A certain absorption of liquid must have taken place by the dry manure, yet this was apparently not sufficient to allow the eggs to hatch; the eggs are actually surrounded by moisture when they are seen hatching. The second point, and an extreme one, was to see whether eggs, when kept under water, will hatch. The droppings were placed in water at a depth of two to three inches and kept at room temperature; two months later faeces so kept were put up in culture tubes, larvae developed and reached maturity. The experiment repeated at a temperature of 32° to 44° F., and extended over a period of two months, gave the same results.

It would thus appear that eggs present in fresh droppings were able to hatch when kept at least for two months in water; when such droppings were microscopically examined whilst they were still under water, the absence of any larvae is remarkable, whereas in the control cultures made at the same time, larvae constantly developed and reached maturity. Nevertheless, differences had been noticed in some of the experiments. Not in all instances did the eggs develop after they were taken out of the water, they apparently had died; and in other instances eggs actually did hatch whilst they were in the water, and the larvae were even able to reach a length up to 900 μ , but a larva was never seen to mature in the water. These differences were particularly noticeable in eggs kept at incubation temperature (98° F.), and at a lower temperature (60° F.). At the higher temperature less eggs remain alive and less larvae reach maturity. This fact finds its probable explanation through the presence or absence of oxygen in the water. At a high temperature absorption of oxygen will take place by the bacteria growing in the liquid droppings.

Summarizing these results, it would appear that eggs retain their vitality in water and can hatch and develop, but cannot reach maturity at a depth of two to three inches. At 93° F., within two days, in no instance appeared mature larvae in the test cultures, showing that larvae of the first and immature second stage placed in water do not develop.

Influence of Low Temperature on the Vitality of Eggs.

Fresh droppings were kept in the ice-box for a period of three months, during which time the daily reading of the thermometer varied from a minimum of 32° F. to a maximum of 46° F. When examined for the first time after three weeks' sojourn in the ice-box, only eggs were seen which did not yet contain the larvae; when put up for culture, mature larvae developed. The same result was obtained at subsequent examinations at intervals of three weeks or a month. The vitality of the eggs was never interfered with, and for the last time after a sojourn of three months, eggs were still alive when put up for culture.

A temperature below freezing point does not seem to destroy the vitality of the eggs. Fresh droppings frozen and kept in a frozen state for ten days retain living eggs.

Influence of the Winter Temperature on the Ova and Development of Larvae in a Moist Medium. (Appendix III.)

These experiments were undertaken with fresh droppings kept in a thermometer screen (Stevenson's screen), and thus protected from the sun. The temperature varied between a minimum of 32° F. in July, and a maximum of 76° F. in the same month. The ova hatched and developed into mature larvae in twenty-two days. In another experiment undertaken in August with the two extremes of temperatures of 28.6 and 84° F., mature larvae appeared after sixteen days. (*Appendix IV.*) As a corollary to the just-mentioned experiments, fresh droppings were placed on open ground and then fully exposed to cold and heat. Mature larvae were noticed on the 25th day after exposure in one instance (in July); the two extremes of temperature were 24.4° F. and 107.6° F., and in fifteen days in the second instance (August), temperature extremes 22.6° F. to 117.6° F. This shows the vitality of the eggs and of the larvae. Under the temperatures mentioned the eggs had been hatching and the larvae were developing and reaching maturity. The winter temperature may retard the development of the larvae, but it does not arrest it.

Longevity of Ova in Droppings under Outside Conditions.

The faeces of infected birds were collected in small heaps, kept in an enclosure protected by wire-netting against access. The surface of the droppings dried up quickly, and thus the eggs in the outer parts were prevented from hatching.

Subsequently, at various intervals, the dried up droppings were examined for the presence of eggs and larvae. They were moistened and immediately placed under the microscope, when larvae of the first stage could be noticed, which, when put up for culture, reached maturity.

This experiment was maintained for the period of fifteen months, when ova and larvae were still found alive. During this time the droppings had been exposed to several soaking rains, which moistened the dry droppings to some extent, allowing a number of eggs to hatch before the dryness arrested further hatching. Droppings kept under natural conditions for fifteen months contained not only live mature larvae, but also small larvae of 210 μ in length (first stage).

The First Ecdysis.

In order to detect the first moulting process it was necessary to keep a constant watch on a given sample of droppings placed under the microscope. Observations were started twenty-four hours after the collection of fresh droppings, which were kept at room temperature varying at that time (December) between 78° to 90° F. Observations were kept up from 7 a.m. until 8 p.m. Numerous larvae had developed; the maximum length reached 480 μ , but none were found in the moulting process. In the morning of the third day observations were continued; the largest larvae had now reached 600 μ . On every slide examined larvae were found actually undergoing the moulting process. Cast skins were noticed measuring from 450 μ to 620 μ in length. On the fourth day no more moulting larvae were found; the culture tube showed many cast skins. On the fifth day the larvae had reached maturity.

The observations were repeated, and again on the third day after collection of the droppings larvae were found in the moulting process. The larvae on which the ecdysis was actually noticed measured 600μ in length. The casting of the skin occupied some time, since a larva which had almost completed shedding its skin was only after ten minutes found to be free of it. A characteristic of the presence of the outer skin is the appearance of wrinkles, whilst the larvae twist about; later the larva is found loose in the skin. Whilst it leaves the skin through the anterior end, its movements are very active.

Similar observations were carried out with droppings kept at a lower temperature, $45-50^{\circ}$ F. Four weeks after the commencement of the first moulting a larva was noticed, 640μ long. Another larva which was in its first skin measured 540μ ; the tail end of the loose skin was empty and measured 100μ . These larvae had reached maturity after another fourteen days.

The result of these observations must be summarized as follows:—

The moulting of larva of the first stage (first ecdysis), under the conditions of room temperature, was observed fifty hours after the commencement of the culture. At this temperature the larvae reached the mature stage in 100 hours. At a low temperature, in which larvae required about six to seven weeks to reach maturity, the first larvae were found moulting after thirty days. Moulting was only observed on larvae which had attained a length of $450-750\mu$.

The rate of growth of larvae varies at different temperatures. Taking the maximum length into consideration at the time of observation, the following notes are of interest:—

At 98° F. the length of 880μ was reached in 65 hours.

At 82.5° F. (average of $77-90$), the length of 900μ was reached in 75 hours.

At 76° F. (room temperature) the length of $800-960\mu$ was reached in 76 hours; but in different observations it varies up to 120 hours.

At 56° F. (average of $48-65$) the length of 800μ was reached on the 17th day.

At 47.5° F. (average of $45-52$) the length of 900μ was reached on the 41st day.

It is evident that the rate of growth increases with the temperature.

Larvae in their First Stage do not Develop in the Stomach of an Ostrich.

Two clean ostriches were used. They were fed daily with the droppings containing larvae, hatched at 90° F. for twenty-four to twenty-seven hours, a period previous to the first moulting. One ostrich obtained $1\frac{1}{2}$ million of larvae and the other $2\frac{1}{2}$ millions. The droppings of the birds so treated were collected and put up for cultures. Such droppings may also contain eggs which had not hatched within the specified time, hence would develop in the culture after passing the ostrich. This fact must be taken into consideration. Eggs do not develop in the stomach, and any subsequent presence of adults would be due to the ingestion of the larvae. The presence of adults would show itself by an enormous increase of eggs in the faeces as the experiment proceeds, and finally could be demonstrated by examining the stomach of the bird killed for this purpose. The result was

that during the period the birds were fed with droppings, only a few larvae appeared in the culture made with the droppings of the drenched birds. After discontinuing the feeding with droppings no more larvae appeared in the culture. The rare occurrence of the larvae justifies the conclusion that larvae of the first stage are not capable of developing in the stomach of an ostrich. They apparently die in the intestines of the ostrich. To control this conclusion, one of the birds was killed 78 days after the commencement of the experiment, and a close examination of the proventriculus showed the absence of any worms.

Larvae of the Second Stage, but Immature, do not Develop in the Stomach of the Ostrich.

Two clean ostriches were fed with larvae of the second stage, viz., larvae from fresh droppings kept at 70° F. for a period of 48-52 hours, the period previously established at which the first moulting would have taken place, or was still taking place. One bird received about 1½ million of larvae in the course of six weeks, the other one 2½ millions in eight weeks.

After the lapse of some time both birds showed a slight infection, indicated by the appearance of rare eggs in the faeces, and similarly as before, this number stood in no proportion to the number of larvae given. Hence it was concluded that larvae in the second stage, as long as they were immature, were not capable of development in the stomach of the ostrich. In order to verify this conclusion one of the birds was killed, and a careful examination with a magnifying glass did not detect the presence of adults.

Mature Larvae Develop in the Stomach of the Ostrich into Adults.

Two clean ostriches were fed with droppings which contained numerous mature larvae. One bird received 500,000 larvae in twenty-four days, and the other one 100,000. After a lapse of thirty-six days in the one, and thirty-nine days in the other, larvae appeared in the culture. Their number increased daily, and they were constantly present at any date subsequently. There can be no doubt that the mature larvae alone are capable of developing in the stomach of an ostrich. To control this conclusion one bird was killed fifty-three days after the last drenching, and an enormous number of adults were present.

Feeding Experiment with Droppings containing Larvae which reached the Mature Stage only a few hours previously.

From the 2/7/13 to the 15/7/13 a clean bird was fed daily with 6-8 oz. of droppings, which had been kept for 96 hours at 90° F.

When examined two months later a heavy infection of the droppings was noted.

Adult Worms taken from the Stomach of a Bird.

Adult worms taken from the stomach of a bird develop in a clean bird when drenched. This contingency cannot be expected to take place under natural conditions. It was carried out, however, to obtain a pure infection with wire-worms, since ostriches taken at random are usually also infected with a sclerostomum living in the caecum, the eggs of which cannot be distinguished from wire-worm eggs in the droppings.

The Mature Larva.

The mature larva is a very active creature in a suitable medium. This is undoubtedly liquid. When droppings are placed in a glass vessel and covered so that the moisture is retained and thrown down in a fine dew on the sides of the glass, the larvae can be seen wriggling in the droplets on the glass walls moving in an upward direction. When kept under these conditions in a usual litre glass cylinder measure they will travel up to the top. Whilst they are in a moist medium they always seem to move about, but they stop all movement when moisture is lacking. Apart from the favourable conditions of moisture, a favourable temperature is also necessary for their activity. This was demonstrated by exposing culture tubes with mature larvae to various temperatures, when it was found that below 38° F. larvae could no longer crawl on to the walls of the glass. Cold, therefore, stops their movements.

The Mature Larva under Natural Conditions.

In order to imitate natural conditions, grass was placed in a few large flowerpots, and these were placed on the ground in a small enclosure to protect them against access of animals. Droppings and mature larvae were placed on the soil of the pots, and during the first fourteen days occasionally watered. Afterwards they were left exposed to rain, sun, and wind. Judging from the agility observed in the glass jars, it had to be expected that the larvae would crawl on to the grasses.

The experiment was started in February. Three months after the experiments were commenced, the grass was examined as to the presence of these larvae by wetting some of the blades that were cut off and washing them in water. Living larvae were found. After another three months the tips of some grasses were washed and living larvae again were found. When the grass had dried up (August and September) some of the dry grass was washed, and again a few living larvae were present. In the course of these experiments it was noted that mature larvae were more frequent on the tips of the grass than on the portion below.

After nine months' observation (November), no more larvae were found on the grass, but in the droppings underneath.

Meanwhile new grass had grown in the pot, and by the end of January it had reached the length of about 16 inches. This new grass was now subjected to examination, when it was found that no larvae were present in the grass above the height of 3 inches, but below it. They were found alive in the original droppings and *on the stems of the new grass*.

Mature larvae were still found alive in the droppings 14 and 17 months after the droppings were placed on the pot. On the grass, however, they were no longer found after 14 months.

The Influence of Complete Dryness on Mature Larvae.

Worm cultures were made in the usual way in a dish. The larvae were collected on the lid and desiccated at various temperatures, 53-61° F., 59-77° F., and 93-98° F. When examined after some months, they were found to revive when moistened. In one instance (desiccated at 93-98° F.) the same larvae were moistened, examined and dried, 4, 5, 7, and 9 months after the first desiccation. The examination after 9 months still revealed a few live larvae.

Mature Larvæ kept in a Moist Atmosphere at the Temperature of the Room.

Droppings were put in test tubes, and these were placed in large stoppered glass jars. The jars contained water, so that the atmosphere of the jar was saturated with moisture. The larvae appeared in due time, and crawled on to the walls of the tubes, where they could easily be watched. After 15 months they were found to be alive, but not so after 17½ months.

How Long do Mature Larvæ Live in Water?

Mature larvae were kept at a low temperature in a tube containing 6 inches of water.

The experiment was discontinued 13 months after commencement, when only a few live larvae could be found.

Exposure of Mature Larvæ to Ice and Cold Water.

Water containing mature larvae was frozen into ice and kept at 16–28° F. for 11 days.

Larvae revived as soon as the ice melted.

The Behaviour of Mature Larvæ when Covered with Soil.

This experiment was planned to find out what live larvae would do when turned under the soil (effect of ploughing on infected ground). For this purpose mature larvae were placed in the centre of the bottom of a glass jar, 8 inches long and 8 inches wide. The vessel was then filled up with moist soil. In the centre of the surface of the soil a glass tube was placed, reaching only a short distance under the soil. After a few days the larvae appeared on the side of the jar as well as on the short glass tube, thus indicating that the mature larvae were actually travelling through the soil on to its surface.

The Migration of the Mature Larvæ.

Cultures in glass jars are most suitable for these observations. (Fig. 13.) When infected droppings are placed at the bottom of a glass jar and care is taken that the liquid does not evaporate, but is deposited as fine dew on to the walls of the jars, then the mature larvae find their best condition to migrate. This migration takes place away from the droppings upwards, and will go as far as the dew is present, provided the jar is exposed to a weak diffused light as obtained on rainy or cloudy days. There is an optimum of diffused light in which the maximum migration is noticed. If the glass jar with the larvae migrating on the walls be exposed to sunlight, or to diffuse strong light, always provided moisture is present, the migration in the opposite direction takes place very rapidly, the larvae hiding where ever there is shade. The larvae are thus negative heliotropic. If the droppings are still sweet, viz., no decomposition having taken place, then the larvae enter into them. This is in particular the case when the droppings are replaced by earth, into which they disappear completely. If the droppings are sour or decomposed, the larvae escaping from the rays of the sun do not enter the droppings, but keep at a distance, they seem to prefer death from sunlight to migrating into decomposed droppings. The larvae are thus also chemotropic. In the experiments not all the larvae so exposed

will die, but those nearest to the glass wall, and thus to the sunlight; the remainder will hide and protect themselves behind the corpses of their mates, forming in clusters. When the evaporation in the glass takes place so rapidly that the larvae cannot escape, but are now exposed to the full sunlight, then they die. That it is the direct action of the sunlight and not of the heat produced by the sun can be demonstrated by placing mature larvae in a glass vessel filled with water; the larvae live in the water under the usual temperature (below 37° C.), but die when exposed to the sun after the lapse of 2-3 days.

These experiments succeed best with fresh cultures. The migration of larvae of older cultures, although still apparent, takes place within shorter ranges.

Do Mature Larvae pass through the Skin of an Ostrich to reach their Ultimate Habitation, the Stomach?

This question had to be answered, seeing that a possibility does exist, since Looss has demonstrated it for *A. kyoostoma duodenale*, and subsequent investigators for a number of other worms.

In December, 1914, on several days, cultures of mature larvae were placed on the skin of an ostrich and allowed to dry; the experiment was repeated in January and February, 1915. On each occasion great numbers of larvae were applied.

The bird was kept under close observation for eight months, and at no time during this period were the faeces found to be infected. This experiment having been carried out under the best of conditions, the conclusion may be allowed, that the mature larvae do not enter the body through the skin.

The Behaviour of Mature Larvae in the Stomach and Intestines of Sheep and Fowls.

This experiment was undertaken after it had been found that mature larvae could artificially be forced to cast their skins. Since it had been realized that hydrochloric acid, or rather the hydrogen ion of any acid, is the actual agency, a casting of the skin in the stomach of an unsuitable host was expected. The question is not without some practical importance.

The experiment was carried out on two lambs and one sheep. They were drenched with mature larvae, and the droppings collected after the drenching were examined, and it was found that of the great number of larvae ingested only a few passed the intestine with their skin not yet cast. The same experiments were carried out on two fowls. The examination of the faeces showed alongside of living larvae a great number of shed skins. Only a few larvae passed the intestine without moulting. One of the lambs and both fowls were killed after the droppings had ceased to contain larvae, and the stomach was carefully searched for nematodes, which were absent in all animals so examined. It appears thus that of a great number of mature larvae only a few pass the intestines.

The Influence of Parasitocides and Disinfectants on Mature Larvae.

A number of drugs were tested both for theoretical and practical purposes, and the results are given hereunder:—

(1) *Lysol*.—A 10 per cent. solution killed mature larvae in 3 minutes; a 2·5 per cent. in 20 minutes; in a 1 per cent. solution a few were still found alive after 1 and 2 hours; when this later experiment was repeated, the larvae after 2 hours were found dead; in a 0·4 per cent. solution after 4–6 hours a few were still alive, only after 10 hours had they all died; in a 0·1 per cent. solution they were still alive after 24 hours.

(2) *Carbolic Acid*.—In a 6 per cent. solution all larvae were dead after 3 minutes; in a 2 per cent. solution after 45 minutes; in a 1 per cent. solution after 1 hour a few were still alive, and after 2 hours all were dead in one experiment and a few still alive in another one; in a 0·4 per cent. after 24 hours a few were still alive, and in a 0·1 per cent. after 24 hours all were alive.

(3) *Picric Acid*.—In a saturated solution (1 per cent.) after 2 hours, about half were killed and after 4 hours a few were found alive.

(4) *Beta-Naphthol*.—In a saturated solution (less than 1 per cent.) after 1 hour the majority of larvae were dead, but after 2 hours only a few were still alive.

(5) *Sulphate of Copper*.—In a 20 per cent. solution after 1 hour about half were still alive, and after 2 hours a few.

(6) *Sodium Arsenite*.—Concentrated solution (less than 10 per cent.). After 10 minutes only a few were found alive.

(7) *Cooper's Dip*.—Concentrated solution. After an hour the majority were alive.

(8) *Sulphuric Acid*.—In a 5 per cent. solution after 15 hours all larvae were dead; in a 1 per cent. solution after 24 hours the majority were dead, and after 48 hours only a few were alive; in a 0·5 per cent. solution after 2 days about half were dead, and after 4 days all; in a 0·2 per cent. solution after 24 hours a few were dead, and after 8 days about half.

(9) *Caustic Soda*.—In a 4 per cent. solution after 15 hours all larvae had dissolved; in a 0·8 per cent. and in a 0·4 per cent. solution after 15 hours all were dead; in a 0·15 per cent. solution half were dead after 24 hours and the other half still alive after 8 days.

(10) *Lime Water*.—Concentrated solution. After 4 hours all larvae were dead.

(11) *Sal-Ammoniac*.—In a 5 per cent. solution after 2 and 4 hours all larvae were found alive, and after 24 hours a few.

(12) *Mixture of 5 per cent. Lime and 5 per cent. Sal-Ammoniac Solution*.—Mixture prepared shortly before use. In less than five minutes all larvae were dead. When repeated some larvae lived for 15 minutes.

(13) *Liquid Ammonia*.—In a 6 per cent. solution after 1 minute all larvae were dead. In a 1 per cent. solution after 30 minutes many were still alive.

(14) *Liquid Ammonia Vapour* (5 per cent. solution).—After 2 hours exposure the larvae were motionless, but revived again. When exposed to vapours of 1 per cent. solution larvae after 3 hours were still alive.

(15) *Turpentine* (Pure).—After 24 hours a few larvae were still alive.

(16) *Paraffin* (Pure).—After 4 days a few were still alive.

(17) *Gasoline Vapour*.—Larvae exposed to gasoline vapours were still alive after 2 hours.

(18) *Carbon Bisulphide*.—Direct contact kills the worms instantaneously. Exposed directly to vapours larvae were killed after 2 minutes. Larvae were placed in water and the vessel containing them was put in a large (1 gallon) vessel into which an ounce of carbon bisulphide was poured. In less than 30 minutes all larvae were dead.

Water saturated with carbon bisulphide killed mature larvae instantaneously. In water containing 0.1 per cent. carbon bisulphide the larvae died in a few minutes.

In interpreting the result of these experiments it must be stated that the object was to find an efficient disinfectant to kill mature larvae outside the host. It would appear that, for practical purposes, solution of carbolic acid, lysol of the usual strength (5–6 per cent.), would be sufficient if applied liberally. Sulphuric and hydrochloric acids would also come into consideration. The acids cause the mature larvae to moult, and the moulted larvae can no longer live when moulting takes place outside the stomach.

Influence of Drugs on Adult Worms when removed from the Stomach.

(a) Worms exposed to vapours of gasoline on examination after 15 minutes and 1 hour were still alive.

(b) Worms exposed to carbon bisulphide at 90° F. were still alive after 15 minutes' exposure. After 30 minutes they were found dead.

(c) Worms exposed to the vapours ensuing from a mixture of 5 per cent. sal-ammoniac and 5 per cent. slaked lime were still alive after 5 and 15 minutes, but no longer after 30 minutes and 1 hour.

Influence of Drugs on the Worms and the Mucus (Stomach Coat) Covering the Surface of Wire-Worm Infected Stomachs.

(a) *Paraffin*.—Pieces of the stomach wall with wire-worms buried in the mucus were placed in paraffin (White Rose) and kept there for 4 hours. After this time the worms were still alive. There was no effect on the so-called stomach coat.

(b) *Petrol*.—No visible effect could be noted on the stomach coat. Worms were still alive.

(c) *Gasoline*.—After 1 hour's contact the worms were still alive.

(d) *Dioxygen* (Pure).—After 1 minute's contact the worms were found alive; they were dead after 5 minutes' contact.

(e) *Dioxygen* (10 per cent.). The worms were still alive after 2 minutes' contact, and dead when examined after 15 minutes.

(f) *Carbon bisulphide*.—Poured into the water 5 per cent. of its volume. Examined after 5 and 15 minutes the worms were found alive.

(g) *Ammonia* (5 per cent.).—After 1 minute, 5 and 31 minutes' contact the worms were found alive.

The mucus adhering to the lining of the stomach showed swelling, became gelatinous, transparent, and detached easily.

(h) *Mixture of 5 per cent. Sal-Ammoniac and 5 per cent. Slaked Lime*.—Examined after 5 and 30 minutes, the worms were found dead. The mucus adhering to the stomach underwent the same changes as under the influence of a 5 per cent. ammonia solution.

These experiments were undertaken to ascertain the effect of drugs in common use on adult worms, the object being to test them on the living animals. The mucous casting is considered to be an obstacle to the approach of otherwise effective drugs. The removal is considered to be essential. Paraffin, petrol, and gasoline are given for this purpose. The results were negative. A solution of 5 per cent. ammonia and the mixture of sal-ammoniac and slaked lime killed the worms after a *relatively short time*. It does, however, not follow that similar results can be expected by administering the same drugs to the living birds. Conditions differ there again. Special experiments were made to clear this question up.

Effect of Various Drugs on the Worm in the Stomach of the Ostrich.

These experiments were undertaken for practical purposes. The drugs tested were those already in use in practice with more or less success (carbolic acid, slaked lime, and sal-ammoniac); drugs which had a good reputation as vermifuges in human medicine, particularly in use against *Ankylostoma duodenale* (thymol, beta-naphthol); and drugs registered in the Veterinary Pharmacopœia as vermifuges (flores koso, kamala, and san-tonin). The judging of the effect was done by the observation of the appearance or disappearance, increase or decrease of eggs, and by putting the droppings up for culture in the days succeeding dosing.

(1) *Carbolic Acid.*

24/11/13. Ostrich No. 44. Dosed with 300 c.c. of 2·5 per cent. carbolic acid solution.

Result.—Eggs were fairly frequent before dosing; after dosing they were still present, but in rare numbers.

24/11/13.—Ostrich No. 54. Dosed with 300 c.c. of 2·5 per cent. carbolic acid solution.

Result.—Numerous eggs before dosing. After dosing they were rare for about 14 days, when they increased again.

22/12/13.—Ostrich No. 44. Dosed with 300 c.c. of 2·5 per cent. carbolic acid solution.

Result.—Eggs which were rare before dosing were rare after dosing.

6/1/14.—Ostrich No. 44. Dosed with 300 c.c. of 2·5 per cent. carbolic acid solution.

Result.—Eggs which were rare before dosing were rare after dosing. The dosing with 2·5 per cent. carbolic acid, or 7·5 grammes per bird, had a slight influence on the worms; it checked the laying of the eggs for a while; it did, however, not kill all the worms.

(2) *Slaked Lime and Sal-Ammoniac preceded 24 hours earlier by a bottle of Paraffin.*

23/11/13. Ostrich No. 42. Dosed with 300 c.c. paraffin.

24/11/13.—Dosed with 1 ounce of slaked lime and 1 ounce of sal-ammoniac in a bottle of water.

Result.—No influence noticed. Eggs appeared as numerous as before.

23/11/13.—Ostrich No. 55. Dosed with 300 c.c. paraffin.

24/11/13.—Dosed with 1 ounce of slaked lime and 1 ounce of sal-ammoniac in a bottle of water.

Result.—No influence noticed. Eggs appeared as numerous as before.

Note.—Contrary to the result with worms treated in a receptacle, no influence was noticed on the worms in their natural habitat.

18/5/15. Ostrich No. 144.

7:30 a.m. Last feed before dosing.

4:30 p.m. Drenched with 400 c.c. paraffin.

19/5/15. 9:30 a.m. Drenched with 40 grammes lime in 400 c.c. water followed by 40 grammes sal-ammoniac in 400 c.c. water.

10 a.m. First feed after drenching.

25/5/15.—Larvae present.

30/5/15.—Larvae very frequent.

(3) *Thymol.*

25/10/13.—Ostrich No. 42. Balled with 6 grammes of thymol in three doses of 2 grammes and in intervals of $\frac{3}{4}$ hour and followed up half an hour later with 250 grammes of mag. sulph. in water.

Result.—For two days following dosing no eggs were found in the faeces. Then they appeared again and increased in number.

4/11/13.—Ostrich No. 54. Balled with 9 grammes of thymol in doses of 3 grammes and in intervals of $\frac{3}{4}$ hour, followed up by 250 grammes of epsom salts after $\frac{1}{2}$ hour.

Result.—For two days following dosing no eggs were found; these eggs appeared again and were very numerous.

10/11/13.—Dosed with 12 grammes of thymol in three doses of 4 grammes and in intervals of $\frac{3}{4}$ hour, followed up by 250 grammes of epsom salts after $\frac{1}{2}$ hour.

Result.—Liquid evacuation with rare eggs after 24 hours. Eggs absent on the 2nd day, present again on the 3rd day and increased after this.

The dosing of the birds with thymol with as much as 12 grammes had but a temporary effect on the worms. It checked the laying of eggs for a few days only.

(4) *Beta-naphthol.*

25/10/13.—Ostrich No. 44. Dosed with 6 grammes of beta-naphthol in 2-gramme doses and in intervals of $\frac{3}{4}$ hour, followed up $\frac{1}{2}$ hour later by 250 grammes of mag. sulph. in water.

Result.—Eggs disappeared for two days, then they appeared again and remained fairly numerous.

4/11/13.—Ostrich No. 46. Balled with 9 grammes of beta-naphthol in 3-gramme doses and in intervals of $\frac{3}{4}$ hour and followed up $\frac{1}{2}$ hour later by 250 grammes of mag. sulph. in water.

Result.—Eggs were never numerous in this bird; they disappeared after dosing for two days and appeared again in rare numbers.

10/11/13.—Ostrich No. 42. Balled with 12 grammes beta-naphthol in doses of 4 grammes and in intervals of $\frac{3}{4}$ hour, followed up $\frac{1}{2}$ hour later by 250 grammes of mag. sulph.

Result.—Liquid evacuation on the 6th day. No dead worms were found. Eggs appeared again.

10/11/13.—Ostrich No. 55. Dosed with 9 grammes of beta-naphthol in 3 grammes and in $\frac{3}{4}$ -hour intervals, followed up by 250 grammes of mag. sulph. after $\frac{1}{2}$ hour.

Result.—Liquid evacuation after 24 hours. Eggs fairly frequent; no worms. Eggs continued to appear in the faeces.

24/11/13.—Ostrich No. 46. Dosed with 12 grammes of beta-naphthol in 4-gramme doses and in $\frac{3}{4}$ hour intervals, followed up by 250 grammes of mag. sulph. after $\frac{1}{2}$ hour.

Result.—Eggs were but rare before dosing. Three days after dosing they were no longer found in the faeces for 12 days, when 1 egg was found. After another 11 days they appeared again in rare numbers to disappear again.

2/12/13.—Ostrich No. 42. Dosed with 10 grammes of beta-naphthol in one dose.

Result.—Eggs were fairly numerous before dosing; they remained fairly numerous after dosing.

2/12/13.—Ostrich No. 55. Dosed with 10 grammes of beta-naphthol in one dose and 24 hours later with 250 grammes of mag. sulph. in a bottle of water.

Result.—Eggs were numerous before and remained numerous after dosing.

23/12/13.—Ostrich No. 57. Balled on three successive days with 12 grammes of beta-naphthol, and 24 hours later with 250 grammes mag. sulph. in a bottle of water.

Result.—Eggs which were rare before dosing were rare after dosing.

23/12/13.—Ostrich No. 58. Balled on three successive days daily with 12 grammes beta-naphthol, and on the 4th day with 250 grammes of mag. sulph. in a bottle of water.

Result.—Eggs which were rare before dosing were rare after dosing.

5/1/14.—Ostrich No. 57. Dosed with 20 grammes beta-naphthol in one dose.

Result.—Eggs which were rare before dosing were rare after dosing.

5/1/14.—Ostrich No. 58. Dosed with 30 grammes of beta-naphthol in one dose.

Result.—Eggs which were rare before dosing were rare after dosing.

The beta-naphthol in enormous doses and in doses repeated, followed by a laxative, had no appreciable effect on the presence of worms. The dosing checked the laying of eggs for a few days, but they appeared again.

(5) *Santonin.*

22/12/13.—Ostrich No. 42. Dosed with 5 grammes of santonin and four hours later 250 grammes of mag. sulph. in a bottle of water.

Result.—Eggs fairly frequent before dosing; they were numerous in the liquid faeces the two succeeding days and remained less frequent than before.

5/1/14.—Ostrich No. 42. Dosed with 10 grammes of santonin.

Result.—Eggs which were not frequent before dosing remained not frequent after dosing.

There was some influence noticeable on the number of eggs before and after dosing, but they did not completely disappear.

(6) *Kamala.*

22/12/13.—Ostrich No. 55. Dosed with 20 grammes kamala.

Result.—Eggs were fairly numerous before dosing and remained so after dosing.

1/5/12.—Ostrich No. 55. Dosed with a decoction of 40 grammes kamala.

Result.—Eggs which were fairly frequent before dosing were fairly frequent after dosing.

Kamala apparently had no effect on the wire-worm.

(7) *Flores Koso.*

22/12/13.—Ostrich No. 54. Dosed with 50 grammes of flores koso and 4 hours later 250 grammes of mag. sulph. in a bottle of water.

Result.—Eggs were fairly frequent before dosing; about 4 days later they were very rare.

5/1/14.—Ostrich No. 54. Dosed with a decoction of 100 grammes of flores koso.

Result.—Eggs which were rare before dosing were rare after dosing.

Some influence was noticeable with flores koso, but no disappearance of eggs could be stated.

(8) *Liquid Ammonia.*

Ostrich No. 144.

12/4/15.—Eggs very frequent.

13/4/15.—Drenched with 300 c.c. liquid ammonia 6 per cent. solution.

15 and 16/4/15.—Cultures negative.

17 and 18/4/15.—Larvae in cultures frequent.

26/4/15.—Drenched with 500 c.c. liquid ammonia 10 per cent. solution.

Bird shows evidence of distress.

1/5/15.—Bird passes pieces of mucosa.

4/5/15.—Larvae present.

Ostrich. No number.

26/4/15.—Drenched with 30 c.c. liquid ammonia in 270 c.c. olive oil.

Three hours later bird passed large amount of mucous matter.

4/5/15.—Larvae present in culture.

18/5/15.—7.30 a.m. Last feed before drenching.

4.30 p.m. Drenched with 400 c.c. paraffin.

19/5/15.—9.30 a.m. Drenched with 500 c.c. liquid ammonia 10 per cent. solution.

10 a.m. First feed after drenching.

20/5/15.—Bird evidently ill.

21/5/15.—Bird dead.

Live adults present at post-mortem.

(9) *Carbon Bisulphide.*

Ostrich No. 59.

12/4/15.—Larvae frequent in cultures.

13/4/15.—Drenched with 30 c.c. carbon bisulphide in 170 c.c. olive oil.

14/4/15.—Diarrhoea and off feed.

15/4/15.—Cultures negative.

- 16/4/15.—Larvae present and fairly frequent.
 19/4/15.—Larvae present and fairly frequent.
 26/4/15.—Dosed with 20 c.c. carbon bisulphide in gelatine capsule.
 3 and 4/5/15.—Larvae frequent.
 18/5/15.—7.30 a.m. Last feed before drenching.
 4.30 p.m. Drenched with 400 c.c. paraffin.
 19/5/15.—9.30 a.m. Dosed with 20 c.c. carbon bisulphide in capsule.
 10 a.m. First feed after drenching.
 25 and 26/5/15.—Droppings smell of paraffin.
 25 to 28/5/15.—Cultures negative.
 29/5/15.—Larvae very rare.
 30/5/15.—Larvae present.
 1 to 5/6/15.—Larvae present.

Conclusions.

The results of the drenching experiments with drugs whose parasitidal action is without doubt when tested in vitro, were but slightly effective or totally ineffective when given to the bird. Although it seems to be possible to check the laying of eggs for a short period, yet only in rare cases could a permanent decrease of eggs be noted, pointing to a decrease of female worms. From the description given of the habitat of the worms in the glands, under the surface of the mucous layer, such disappointing results were not unforeseen and belong to the practical knowledge of the ostrich farmer.

The Habitat of the Wire-worm.

Anatomical Considerations. (Figs. 14 and 15.)

The proventriculus of the ostrich is the habitat of the parasitic stages of the wire-worm. It represents the continuation of the lower end of the oesophagus, its position is immediately above and behind the ventriculus, forming a curve from the top to the back of this part of the stomach into which it opens with a wide opening and narrowed at its entrance by a constriction. The continuation of oesophagus into the proventriculus is a gradual one; there is also a shallow constriction in the upper wall of the proventriculus, somewhat in front of the large curvature. In front of this constriction the proventriculus bulges out slightly, forming a shallow caecum. This formation is absent in some birds.

In the mucosa four different regions can be distinguished:—

- (1) The zone bordering on the oesophagus.
- (2) The glandular zone under the curvature of the proventriculus.
- (3) The zone on the sides both connected by the small ventral curvature.
- (4) The connecting zone leading from the proventriculus to the ventriculus (the intermediary zone).

The first zone is represented by a smooth cuticular mucosa consisting of a fairly thick stratum of simple straight tubular mucous glands, widened at the bottom, narrowing towards the opening leading to the surface. The lumen is lined with large transparent cells. The propria mucosae is fairly thick and contains a number of globular lymph follicles pushing their way

between the tubular glands and the epithelial stratum reaching the surface of the mucosa. On some places these lymph follicles are numerous and closely packed together, whilst in others a diffuse infiltration of the propria with lymph cells is prevalent (tonsilla).

The glandular zone is raised above the surrounding surface and has an areolated appearance, each area being sharply defined by a polygonal border line, separating the various glands. The middle of each areola contains a small pit, the opening of the gland; its surrounding is slightly raised, so that the surface has a somewhat warty appearance. The mucosa of this portion consists of two kinds of glands, viz., of closely packed tubular glands, forked and branched in their course. Their lumen and the surface is covered with a fairly thick layer of mucus (glandulae superficiales mucosae). The second kind of glands seem to be formed by an inversion of the tunica propria downwards, forming a bag. It is divided into a number of chambers, each chamber corresponding to a lobule with its own lumen, and the various lumina joining up to a common exit leading on to the surface, forming the pit of the areola. The tubuli are placed radiating from the lumen. On cross-section, viz., at right angles to the lumen of the lobule, the tubuli form a network; cross branches connect the opposite walls. The tubuli consist of cubic epithelial cells, which on the entrance into the lumen become cylindrical in shape. The nuclei are placed at the bottom of the cells, and a margin of protoplasm borders the surface. These glands correspond to the glandulae propriae profundae. In the periphery of the glandular region these glands consists of a few lobuli only; in the centre the number of the lobuli increases and then larger glands are formed.

The third zone occupies the largest portion of the stomach, fully four-fifths. Its mucosa is folded longitudinally, the folds lying more or less parallel. It is, however, not a true folding of the mucosa: the folds are represented by longitudinal ridges of the propria, which is covered with a stratum of tubular glands. The cubiform cells of these tubuli contain a dark stainable nucleus at the bottom of the cells; they are placed at about the same level. Mucus is found in the tubuli, but particularly on the surface, and here in a fairly thick layer. These ridges run close together in the portion abutting on the glandular region, and between them crypta are found, in which the mucosa consists of forked and branched tubuli as on the surface of the glandular portion itself.

The fourth portion is again smooth. It consists of a stratum of tubuli, which are short. The surface is covered with a thick layer of a substance resembling that of the ventriculus (wrongly named horny substance).

The Situation of the Wire-Worms. (Figs. 16, 17, 18, 19.)

The parasitic stages of the wire-worm are found either in the lumina of the deep glands or lying between the superficial tubular glands of the adjoining region (the third zone). In sections through the glandular portion taken on the third day after infection, they were not yet found, but in sections taken on the sixth day; it is possible that they enter the glands only after the moulting process (casting of the third skin). They are found here in all their later stages. In a stomach of a killed bird clusters escape

through the pit of the gland, when slight pressure is exercised. They are equally frequent in the depths of the mucosa of the adjoining third zone. In the fresh stomach they can be demonstrated by scraping the mucous layer off the mucosa, particularly in the crypta between the folds. They are not found, or only very rarely, on the surface. It appears that they proceed to the surface when the adult stage reaches maturity. From about the 24th day and subsequently the effect of the presence of the worm becomes noticeable, the mucus is detached in parts, and a hæmorrhagic infiltration of the mucosa becomes visible. When the worms are mature and numerous, then the mucosa has a swollen appearance; it is thickened and covered with a copious mucus. This condition is called "vrotmag" (rotten stomach) by the Boers. The sequel to this condition is anaemia and poverty of the birds.

From this description it becomes evident that drugs can hardly reach the well-protected younger stages of the wire-worm.

Practical Deduction for the Rearing of Ostriches Free of Wire-Worm.

For our purposes the following guiding principles may be laid down:—

(1) An ostrich, once infected with wire-worm, will remain infected for a long period, perhaps for years, and during this time it will be a constant source of infection of the pasture on which it drops its faeces.

(2) There is as yet no treatment which with certainty will expel all wire-worms from an ostrich, and to judge from the position of the wire-worm in the glands and the mucosa, it is not likely that such will be found.

(3) Once a pasture or a run is infected with wire-worm it can remain infected for a long time, both eggs and larvae maintaining the infection.

(4) The fresh droppings are not infective, and only become so after a while, which is shorter in hot weather and longer in cold weather.

(5) Only the species ostrich can act as a host for the worm.

(6) Ostriches can stand an enormous infection of wire-worm, provided they are well fed (feed both worms and birds).

(7) Ostrich chicks do suffer from wire-worm infection even when well fed, and may succumb from larval infection, viz., before the larvae have reached the adult stage.

(8) By means of worm-cultures made from droppings of the bird, it can be detected whether a bird is infected with wire-worm. This culture can be made in a simple way by placing the fresh droppings into a wine glass, covering it with a suitable lid to prevent evaporation of the moisture. After a few days the larvae can be seen crawling on the wall of the glass and can be recognized by the naked eye. Their number present gives an indication to what extent a bird is infected.

From these notes it must be concluded:—

(1) That wherever ostriches have been running the pasture is infected, and wherever the pasture is infected the birds will be so too.

(2) It is accordingly not advisable to rear young ostriches on ground where old ostriches have been running and feeding.

(3) In the case of old infected ostriches but little can be done except to dose them with drugs as hitherto applied (carbolic acid, slaked lime, sal-ammoniac, etc.). Good feeding is essential.

(4) On ostrich farms, where clean ground is no longer available, clean runs for young birds can be prepared and the birds can be reared in such runs until they have reached the age when wire-worms are no longer so dangerous to the birds. The cleaning of the run is best carried out by removing the surface of the ground to the depth of about three inches. Use can be made of disinfectant, but this is less certain.

(5) The chicks must then be fed with the usual foodstuffs grown on land over which no birds have been running.

(6) The runs of the chicks must be so placed that infected birds do not come in contact with them, and no flooding takes place.

(7) These runs must be stocked with chicks reared in an incubator, or chicks removed from the nest immediately after hatching.

(8) When hens and chicks are kept together, it would be advisable to clean the runs at least every 48 hours (better every 24 hours), by picking up all faeces of the adult birds, which is best done by daily changing the birds from one run to another, whilst the one is cleaned.

(9) For the purpose of rearing ostriches no large paddocks are required, small runs will be sufficient. We found a run of 100 by 50 feet quite sufficient and successful.

(10) Chicks reared in this way will also be free of other intestinal worms (*Sclerostomum* and Tapeworm).

(11) An effective contral can be carried out by means of the glass cultures as described before.

Experiments to Show that the Suggestion can be Carried Out in Practice,
(Figs. 20 and 21.)

These were done at Onderstepoort, and on ground where previously no ostriches were kept. A number of runs were closed in with the usual fencing, with a gangway separating the runs; at the back of the run was a small house to shelter the chicks at night or during adverse weather. The ground was levelled out and gravelled. Chicks were placed into them when a few weeks old, having previously been kept in a smaller pen. The chicks were hatched in an incubator.

Breeding birds were kept in separate paddocks placed some distance away, and so arranged that no contamination of the chick runs was possible. The size of the paddocks was the one usually adopted for this purpose. Our experience shows that hens will lay eggs in a much smaller run; a cock and two hens were kept in a run as described above, and eggs were obtained as regularly as from the paddocks. Under the conditions described we reared ostriches hatched in September, 1912, and kept them until September, 1914; and again from October, 1913, until December, 1914, a total number of 100 birds. Every month the droppings of the birds were collected and put up for culture; at no time could wire-worm eggs be detected, or young larvae be reared. The birds were fed in the usual way: mealies, green lucerne, chopped prickly pear according to the season, with a supply of bone and grit. The birds represented at all times a picture of health.

Experiments to Show that a Run can be Cleaned of all Infection.

A run in which infected birds had been kept for a period of one year, during which time it was never cleaned, was utilized for this purpose. Then the surface soil to the depth of three inches was removed and filled up with sand and gravel taken from the Aapies River bed. A number of clean birds (9) were then placed in this run. Their droppings were collected once a week and put up for culture test. During a period of eight months (December to August), the control was strictly kept up, and at no time could infected birds be detected in the run.

Experiments to Show whether the Disinfection with 5 per cent. Sulphuric Acid is sufficient to Destroy the Infection.

In place of removing surface soil it was sprayed on three occasions with 5 per cent. sulphuric acid solution. Clean birds were placed into it. It was found that the birds contracted a slight infection under these conditions.

Experiments to Show whether it is Possible to Keep Infected and Not Infected Birds in the Same Run provided the Run is Cleaned every 48 Hours.

Twelve infected birds and six clean birds were kept together and the run was brushed out every other day, care being taken to remove all the droppings. The control birds were once a week locked up in the house, and their droppings were collected and put up for culture. During the eight months of observation no infection of the clean birds took place. It must, however, be stated that the twelve infected birds contained only a slight infection.

Conclusion.

The solution of the wire-worm problem does not lie in the drenching of birds with drugs, but in the rearing of birds free of parasites. That this can be done our experiments should clearly show.

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APPENDIX I.

- 23/7/12.—Fresh droppings placed in ice-chest. Microscopical examination shows ova fairly frequent.
- 1/8/12.—Temperature, 45–50°. Majority of eggs in embryo stage. No larvae observed.
- 6/8/12.—Temperature, 46–47°. Live larvae fairly frequent. Largest, 240–400 μ . A few dead.
- 12/8/12.—Temperature, 45–49°. Live larvae fairly frequent. Largest, 500–550 μ . Majority, 400–500 μ .
- 17/8/12.—Temperature, 45–50°. Live larvae fairly frequent. Largest, 550–600 μ . Majority, 450–550 μ .
- 23/8/12.—Temperature, 45–50°. Live larvae fairly frequent. Largest, 720 μ . Very few dead (smallest only). Majority, 560–640 μ . One in moulting stage, length 640 μ ; another moulting, tail-end of sheath protruding 100 μ . One moulting, length 560 μ ; length of outer skin, 680 μ .
- 28/8/12.—Temperature, 46–52°. Largest larvae, 880 μ . Majority, 700–800 μ . Larvae moulting (600 μ). None on wall of tube.
- 3/9/12.—Temperature, 46–50°. Majority, 800–900 μ . None on wall of tube.
- 9/9/12.—Temperature, 45–49°. Larvae on wall of tube very rare. Mature larvae.
- 15/9/12.—Temperature, 44–47°. Larvae on wall of tube rare. A decided majority of larvae alive; only smaller ones dead.
- 15/9/12.—Culture placed with corresponding one (temperature, 43–48°) at a temperature of 94°. Larvae frequent on wall of tube.

APPENDIX II.

Fresh droppings were desiccated with calcium chloride at room temperature.

5/3/12.	Desiccation commenced.				
26/3/12.	1st test made.	Result: Larvae frequent.			
4/4/12.	2nd " "	"	"	"	present.
7/5/12.	3rd " "	"	"	"	present.
6/6/12.	4th " "	"	"	"	very rare.
8/7/12.	5th " "	"	"	"	rare.
5/9/12.	6th " "	"	"	"	rare.

WEIGHT OF DROPPINGS.

		Loss.
5/3/12.....	18.6 grammes.	11.3 grammes.
20/3/12.....	7.3 "	
20/3/12.....	5.90 "	0.03 "
26/3/12.....	5.87 "	
26/3/12.....	4.50 "	0.03 "
4/4/12.....	4.47 "	
4/4/12.....	3.50 "	0.01 "
7/5/12.....	3.49 "	
7/5/12.....	3.00 "	0.00 "
6/6/12.....	3.00 "	
6/6/12.....	2.50 "	0.00 "
8/7/12.....	2.50 "	

APPENDIX III.

Fresh droppings in culture tubes kept in a thermometer screen, exposed to cold, but protected from the sun.

Date.	THERMOMETER READINGS.		Date.	THERMOMETER READINGS.	
	Minimum.	Maximum.		Minimum.	Maximum.
12/7/12	32.0	70.6	3/8/12	36.2	77.0
13/7/12	32.8	70.0	4/8/12	43.2	67.0
14/7/12	39.0	72.6	5/8/12	33.8	68.6
15/7/12	42.4	65.2	6/8/12	31.8	65.0
16/7/12	46.8	66.8	7/8/12	28.6	67.8
17/7/12	38.6	61.2	8/8/12	31.6	67.2
18/7/12	39.0	70.6	9/8/12	31.6	69.0
19/7/12	42.2	70.2	10/8/12	35.8	72.2
20/7/12	31.8	72.6	11/8/12	36.2	71.8
21/7/12	—	—	12/8/12	36.0	70.0
22/7/12	36.2	76.0	13/8/12	38.6	73.4
23/7/12	35.0	74.6	14/8/12	38.6	76.6
24/7/12	33.8	74.4	15/8/12	39.2	77.6
25/7/12	35.8	73.6	16/8/12	44.4	79.6
26/7/12	35.0	72.0	17/8/12	42.4	82.8
27/7/12	40.4	71.6	18/8/12	48.0	84.2
28/7/12	34.8	62.2	19/8/12	42.2	80.0
29/7/12	36.8	64.0	First encysted larvae.		
30/7/12	37.8	65.2			
31/7/12	37.6	72.0			
1/8/12	30.8	74.6			

First encysted larvae.

12/7/12	Commenced.	3/8/12	Commenced.
1/8/12	First encysted larvae.	19/8/12	First encysted larvae.
3/8/12	Encysted larvae fairly frequent.	23/8/12	Larvae rare.

APPENDIX IV.

DATE.	THERMOMETER READINGS.		DATE.	THERMOMETER READINGS.	
	Minimum.	Maximum.		Minimum.	Maximum.
12/7/12	24.4	99.6	3/8/12	25.6	101.4
13/7/12	25.4	101.4	4/8/12	39.4	93.6
14/7/12	31.8	102.8	5/8/12	25.8	87.6
15/7/12	36.6	90.0	6/8/12	22.6	99.4
16/7/12	45.6	90.4	7/8/12	22.0	102.4
17/7/12	34.6	68.6	8/8/12	23.0	101.6
18/7/12	31.6	103.8	9/8/12	24.6	101.6
19/7/12	33.4	96.8	10/8/12	27.8	106.2
20/7/12	26.0	103.0	11/8/12	27.8	110.2
21/7/12	—	—	12/8/12	29.4	102.8
22/7/12	28.2	106.4	13/8/12	32.4	97.0
23/7/12	27.6	99.6	14/8/12	32.6	102.6
24/7/12	28.0	100.6	15/8/12	32.6	103.6
25/7/12	29.8	101.2	16/8/12	37.0	111.0
26/7/12	27.4	105.0	17/8/12	34.0	117.6
27/7/12	33.2	91.2	First encysted larvae.		
28/7/12	26.0	95.4			
29/7/12	28.6	89.8			
30/7/12	30.8	95.8			
31/7/12	28.2	99.8			
1/8/12	23.6	107.6			
2/8/12	22.6	92.6			
3/8/12	25.6	101.4			

First encysted larvae.

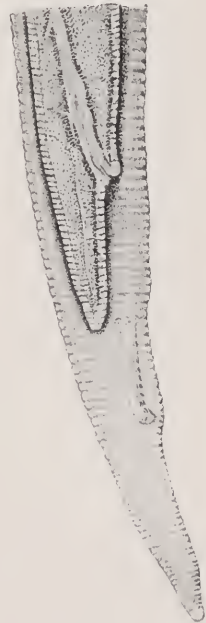
12/7/12	Commenced.	13/8/12	Commenced.
3/8/12	First encysted larvae.	17/8/12	First encysted larvae.
7/8/12	Encysted larvae rare.	21/8/12	Encysted larvae rare.

DESCRIPTION OF PLATES: *Tricho-Strongylus douglasi*.

- Drawing* No. 1. Posterior end of 1st larval stage. Magnified 666+.
- „ No. 2. Posterior end of 2nd larval stage. Magnified 666+.
- „ No. 3. Posterior end of larva in 2nd ecdysis. Magnified 666+.
Loose skin shows tail-end of 2nd larval stage and contains larva of 3rd stage.
- „ No. 4. Posterior end of 4th larval stage; female. Magnified 666+.
Immediately after 3rd moult.
- „ No. 5. Posterior end of larva in 4th ecdysis. Magnified 333+.
Loose skin shows tail-end of 4th larval stage and contains young male in adult stage.
- „ No. 6. Loose skin contains young female in adult stage. Magnified 333+.
- „ No. 7. Posterior end of mature female adult. Magnified 333+.
- „ No. 8. Posterior end of mature male adult. Magnified 333+.
- Photo* No. 9. Mature male adult. Magnified 40+.
- „ No. 10. Mature female adult. Magnified 40+.
- „ No. 11. Ova in morula stage. Magnified 175+.
- „ No. 12. Mature free larvae. Magnified 80+.
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- „ No. 14. Positions of ventriculus and proventriculus of the stomach in the ostrich.
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- „ No. 17. Cross-section of a deep propria gland. Magnified 15+.
- „ No. 18. Cross-section of a fold. Magnified 40x.
- „ No. 19. Cross-section of a deep propria gland. Magnified 15+.
Bird infected twelve days previously.
- „ No. 20. Experimental paddocks for the rearing of ostriches at Onderstepoort.
- „ No. 21. Chicks reared free of infection.
- „ No. 22. Kennel with cement floor, to prevent reinfection during experiment.

*Fig. I.**Fig. II.**Fig. III.**Fig. IV.**Fig. V.*

Wire-Worm in Ostriches,

*Fig. VI.*

L. Theiler & W. Robertson.

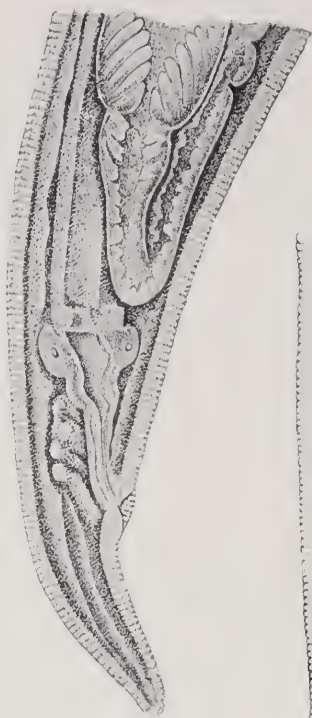
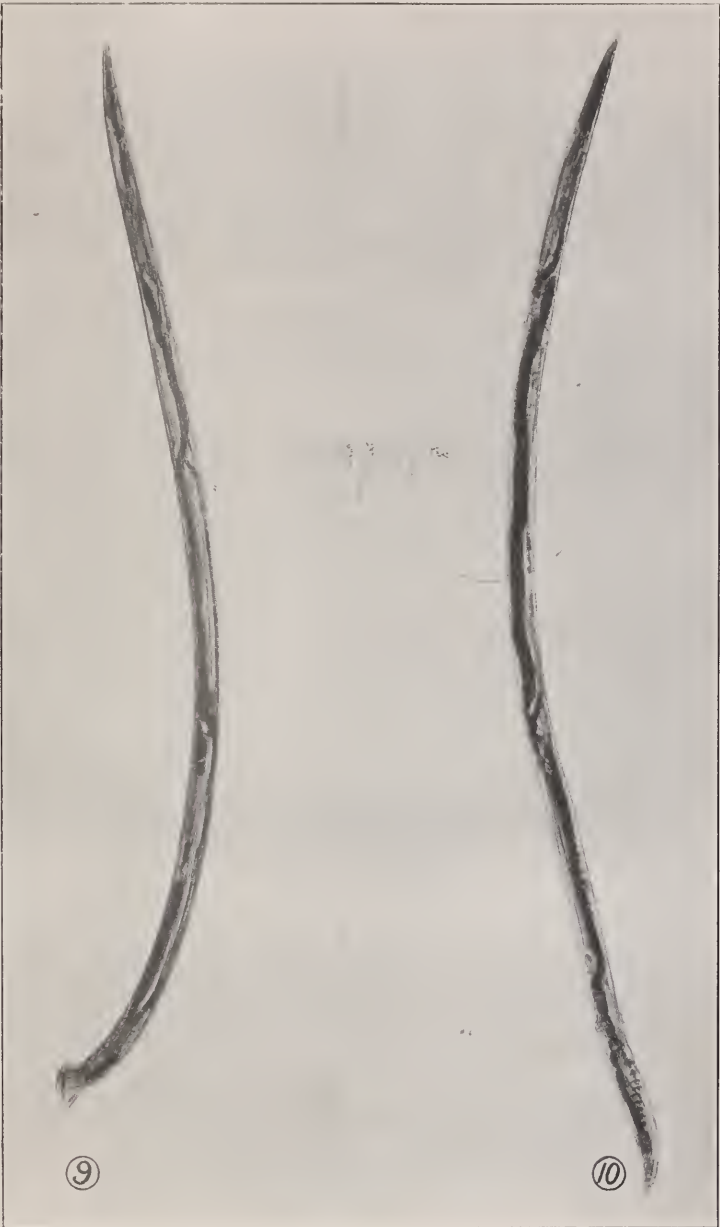


Fig. VII.

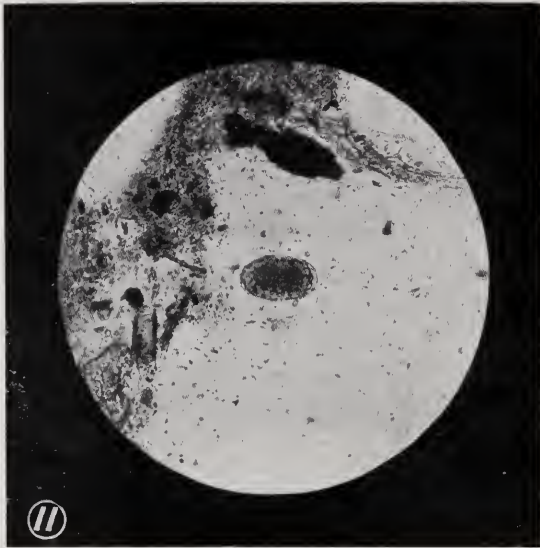


Fig. VIII.



Wire-Worm in Ostriches.

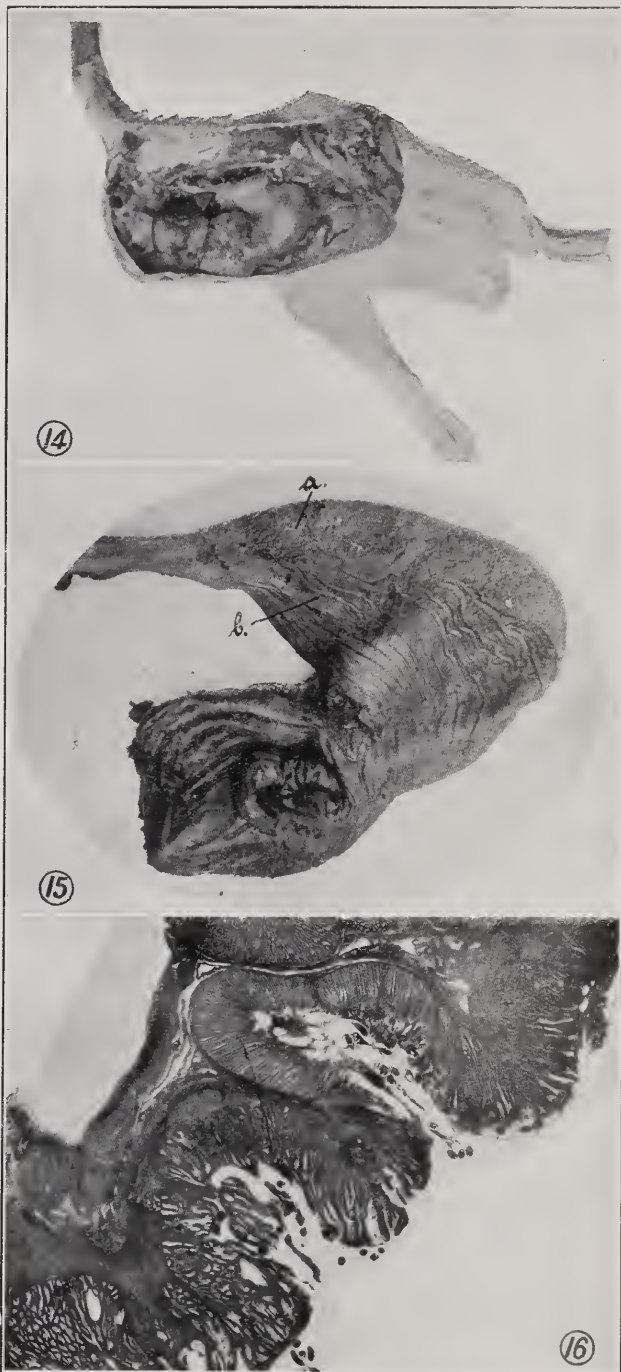
A. Theiler & W. Robertson.





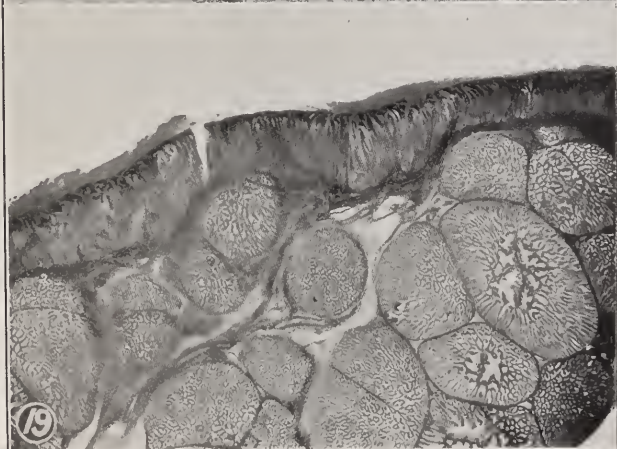
Wire-Worm in Ostriches.

A. Theiler & W. Robertson.



Wire-Worm in Ostriches.

A. Theiler & W. Robertson.





Wire-Worm in Ostriches.

A. Thelker & W. Robertson.



The Anatomy and Life-History of the Haemonchus Contortus (*Rud*).

BY

Dr. FRANK VEGLIA,

Veterinary Research Laboratories, Onderstepoort.

The Anatomy and Life-History of the *Haemonchus Contortus* (Rud).

By Dr. FRANK VEGLIA, Veterinary Research Laboratories,
Onderstepoort.

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INTRODUCTION.

THE experimental work forming the subject of this paper was commenced in 1911 at the instigation of Dr. (now Sir Arnold) Theiler, Director of Veterinary Research, with the object of obtaining accurate data on which a scientific prophylactic treatment could be based.

In the first instance, the anatomy, morphology, and cycle of development of *Haemonchus contortus* was studied, and experiments are now being undertaken from the point of view of medicinal treatment.

FREE LIFE.

THE EGG.

The methods of examination applied were as follows—

1. Examination of fresh faeces, placed directly under the microscope.
 2. Sedimentation method, in which the faeces are emulsionized with water, allowed to precipitate, and the sediment collected.

3. The sieve method, which is an improvement on method No. 2, consisting in using a sieve of suitable mesh, whereby the coarser particles are retained, and the eggs found in the sediment.

4. The centrifugalizing method, which is another variation of the two last-mentioned methods, consisting in centrifugalizing the sediment whereby the eggs (being heavier than the particles of faeces) remain at the bottom of the tube, and can easily be collected by means of a pipette.

5. The comminution method, consisting in a combination of the above methods suggested by Mr. Maurice Hall. The method can be described briefly in his own words. “. . . After having been broken up, the faeces are poured through a set of six brass sieves. The sieves have a mesh aperture ranging from 3 mm. in the largest to about one-fourth of a millimetre in the smallest. . . . The sieves are nested in the order of mesh aperture, with the coarsest on top, and placed in a large porcelain evaporating dish or in a large crystallization dish. The faeces are poured into the top screen, and pass through the screens to the evaporating dish, particles of different sizes being held by different screens. . . . Tap water or normal salt solution is poured in the upper sieve until the water stands in the evaporating dish at a level above that of the bottom of the upper sieve. This sieve is lifted and shaken a little until the fine matter has passed through. It is then lifted out and put in a large crystallization dish half full of water or salt solution and the matter it contains examined on the screen or washed into the dish. . . . The sediment left in the evaporating dish after removing the finest sieve is poured on to a screen of Miller's silk bolting cloth with a mesh aperture of 0.117 to 0.134 mm., and the finer particles washed through into a tall jar.” The sediment is then centrifugalized, and in this way the eggs are obtained in a concentrated residue of faeces which can be poured on a microscopical slide for examination. The material thus obtained is also suitable for preservation after having been fixed by one of the usual methods.

A number of other methods have been suggested by Bass, Garrison, Wellman, and Teleman, in which chemicals are used to dissolve the soluble constituents of the faeces, but these methods are not to be recommended, as the chemicals may be injurious to the eggs, more especially if the eggs are to be used for purposes of cultivation. I tried practically all the above methods, but abandoned them in favour of another one which consisted in (1) breaking up all the faeces by means of a spoon and diluting with enough water to bring the material to a semi-liquid consistency, (2) passing through a brass sieve with a mesh aperture of .5 mm. to 1 mm.; (3) sedimenting in a jar, removing the supernatant liquor, and washing with water by three successive sedimentations;

(4) centrifugalizing the sediment and collecting the eggs with a pipette. The sediment was found to be particularly suitable for cultural experiments.

For working with one particular worm, collection of material in the way indicated is only of value if the animal contains a pure infection of that species, but under natural conditions this is most frequently not the case, and then recourse has to be taken to the study of eggs freshly obtained from females. For this purpose, live females were obtained, from an animal only recently dead, placed in tepid water, and kept in an incubator. The worms were from time to time transferred to new Petri dishes where they subsequently laid eggs; in the case of *Oesophagostomum columbianum* it was found possible to keep the worms alive for twelve to twenty hours. The eggs thus obtained can be used for the purpose of cultivation or for microscopical examination. The method of Looss, which can be summarized as follows, was utilized for the preservation of eggs:—70 per cent. alcohol at a temperature of 60° C. is poured over the egg. The following day the alcohol is changed and replaced by 80 per cent, and later 90 per cent. In order to make the eggs transparent they are removed from the 70 per cent. alcohol to 70 per cent. alcohol plus 5 per cent. glycerine. The alcohol is then allowed to evaporate either at normal temperature or at 56° C. Some more liquid is added and allowed to evaporate until the eggs are finally in pure glycerine. Eggs can be preserved in this medium.

Morphology of the Egg.

An egg of *Haemonchus contortus* is oval, with one side frequently more curved than the other, the poles being unequal, one being usually less convex than the other. The average size is $70.9\mu \times 45.9\mu$; a common minimum size is $66.5\mu \times 43.3\mu$ and a frequent maximum is $79\mu \times 46.6\mu$.

In fresh droppings the eggs have a transparent shell slightly yellowish in colour with a thickness of about 1 micron and sometimes even less. The yolk of the egg apparently occupies the whole of the shell, but on both poles there are frequently empty spaces noted, 3 to 4 micron diameter. In some cases a polar body is detected in one or both of these spaces. The yolk is surrounded by a hyaline substance. The yolk is segmented, and in faeces freshly examined 24–26 segments or blastomeres can be counted. These blastomeres are homogenous, measure 12 microns in diameter, are granular in appearance with nuclei appearing as a faint spot in the centre of each blastomere. When an egg is examined from the right-hand side, eight blastomeres are found superficially in one upper layer. Below this is situated another exactly similar layer. Sandwiched between these two, and partly projecting over their margins, is a third layer also with eight blastomeres. Fig. VII may be taken as representing the way in which the intermediate layer shows up below the uppermost eight blastomeres.

The yolk frequently touches the hyaline membrane, whilst in other cases there are empty spaces, varying in size, between the two. An egg freshly laid by the female is mostly found to be in the four-cell stage. It is exceptional to find eggs in three or even two cell stage which, under suitable conditions, continue their development. On examining the female, eggs in the one-cell stage are found in the distal portion of the uterus

(Fig. 1-2), in the median portion two and three cell stages (Fig. 3-4), and in the proximal one, four-cell stages (Fig. 5).

In the stomach of the host eggs at the six, seven, and eleven cell stages (Fig. 6) may be found and, exceptionally, also at the "morula stage." No eggs beyond the morula stage are found in the intestinal tract even in cases of constipation. The larger majority are in earlier stages. It is possible that oxygen is necessary for a further development from the morula stage, and as oxygen is practically absent in the intestines all eggs are thus found only in stages up to the morula. This opinion is based on the observation, that if infected faeces are stirred up with water and placed in culture, only the eggs in contact with the air hatch. Hatching is observed to take place outside the body at a temperature higher than that found in a normal sheep, and the body temperature cannot therefore be held responsible for the inhibition of development.

Formation of the Embryo.

The embryonic development of *Haemonchus contortus* does not differ from that described by other authors in the case of other strongylidae. The duration of the embryonic period varies according to temperature and media, even in eggs obtained from the same source.

The following notes refer to certain investigations undertaken into the development of the embryo in eggs kept at 26° C. in a dark place under favourable conditions of moisture.

Initially.—The eggs used consisted of batches obtained from faeces and found to contain 11-26 blastomeres, together with batches in the morula stage from sources indicated in the table below.

Four Hours Later.—A number of the eggs are in the advanced morula stage (Fig. 9). The hyaline sac is filled with a number of blastomeres, averaging from about 6 μ in diameter. On the wider blunter pole of the two, the morula contains a depression, and running through the centre a dark longitudinal stripe seems to indicate the initial stages of the alimentary canal. The whole egg is occupied by the embryo, but on both poles there is an empty space equal to about one-tenth of the length of the egg. In breaking the egg in this particular stage, I was able to count ninety blastomeres. There are only a few eggs to be found so far advanced.

Six Hours Later.—The embryo is now at the tadpole stage (Fig. 10-11), the anterior end being about twice as thick as the rest of the body and the thicker part slightly curved. A depression representing the mouth is now well marked, and it is possible to detect some movements on the part of the embryo.

Eight Hours Later.—The embryo is now twice the length of the egg (Fig. 12), and shows a few structural details. The anterior end is broader, and somewhat conical. A buccal cavity is recognizable by its cylindrical shape. A strand of black cells can be distinguished as the initial stage of the oesophagus and the intestine. The movements of the embryo are now frequent. At this period of observation numbers of eggs are also in the tadpole stage, whilst many others are still in the morula stage.

Ten Hours Later.—In some cases the embryo attains three times the length of the egg (Fig. 13), whilst in a few others it is four times as long. There are still some eggs in the same batch in the morula stage. Embryos are frequently found to be curled up in the shape of a figure 8 and show frequent, or often constant, movements (Fig. 14). At this stage the shell is still fairly resistant, as is proved by the fact that pressure on the cover glass frequently leaves the shell intact but breaks the embryo inside. The shape of the body is now cylindrical, the head is conical, and the tail long. The mouth cavity is well marked by two longitudinal lines, each terminating posteriorly in a point. The œsophagus has the rhabditoid appearance, and the so-called "teeth apparatus" is distinct. The chyle intestine contains blackish, slightly transparent granules. The lumen, although hardly visible, is narrow and spiral-shaped. The rectum is long and wide. Around the constriction of the œsophagus is a transparent ring which can be recognized as the primordium of the nerve ring. Some embryos obtained from eggs that were broken accidentally were measured, and the length was found to be $280\mu \times 17\mu$ thickness, whilst the egg itself measured $73.29\mu \times 43.27\mu$. At this period of observation a few free larvae are occasionally found.

Twelve Hours Later.—The movements of the embryo are now more marked, but no further development seems to have taken place.

Fourteen Hours Later.—About 25 per cent. of eggs are found to have hatched.

Seventeen Hours Later.—About 50 per cent. of the total number of eggs are now found to have hatched. The remainder hatch slowly, and by the 48th hour only a few eggs have not hatched.

In cultures made in a liquid medium the majority of eggs hatched from the 14th to the 17th hours, probably owing to the more uniform conditions prevailing.

Hatching of the Embryo.

I am under the impression that the embryo breaks loose from the egg owing to want of room and food within the shell. The young larva can be seen to persistently move its head against the inner side of the wall; after several futile attempts it changes its position and repeats the pushing movement of the head. This process is repeated several times until the embryo finally succeeds in making a hernia-like protuberance which bursts and allows the young larva to escape.

Table to show Period of Hatching in Different Media.

No. of Experiment.	Temperature.	Medium.	Where Eggs were Obtained.	Time in Hours.	REMARKS.
1	27° C.	Faeces	Faeces	9	No larvae.
				14	80 % young larvae hatched.
2	35° C.	"	"	13	No larvae.
				15	23 % young larvae hatched.
2a	35° C.	"	"	15	No larvae.
				18	30 % young larvae hatched.

No. of Experiment.	Temperature.	Medium.	Where Eggs were Obtained.	Time in Hours.	REMARKS.
9	35° C.	Decoction of Faeces	Laid by female :		
			2nd lot....	18	80 per cent. larvae hatched.
13	35° C.	..	1st lot....	17	Some young larvae hatched.
				19	35 per cent. larvae hatched.
				21	Nearly all larvae hatched.
14	35° C.	..	2nd lot....	18	60 per cent. of larvae hatched.
				22	Nearly all larvae hatched.
15	35° C.	..	1st lot....	20	10 per cent. larvae hatched.
			2nd lot....	18	20 per cent. larvae hatched.
16	35° C.	..	1st lot....	19	50 per cent. larvae hatched.
			2nd lot....	17	50 per cent. larvae hatched.
			3rd lot....	15	5 per cent. larvae hatched.
18	35° C.	..	1st lot....	22	Few larvae hatched.
			2nd lot....	16	No larvae.
				17	20 per cent. larvae hatched.
19	35° C.	..	1st lot....	22	2 per cent. larvae hatched.
			2nd lot....	15	60 per cent. larvae hatched.
			3rd lot....	14	60 per cent. larvae hatched.
20	35° C.	..	1st lot....	24	No larvae.
			2nd lot....	16	2.3 per cent. larvae hatched.
21	35° C.	..	2nd lot....	16	50 per cent. larvae hatched.
22	35° C.	..	Faeces	13	Some larvae hatched.
				16	90 per cent. larvae hatched.
23	35° C.	11	No larvae. Eggs in advanced stage.
		Faeces	..	11	No larvae.
		Decoction of faeces	..	14	30 per cent. larvae hatched.
		Faeces	..	14	50 per cent. larvae hatched.
		Decoction of faeces	..	19	60 per cent. larvae hatched.
		Faeces	..	19	95 per cent. larvae hatched.
24	35° C.	Decoction of faeces	Stomach contents	19	60 per cent. larvae hatched.
		Faeces	..	19	90 per cent. larvae hatched.
		Decoction of faeces	..	21	All larvae hatched.
		Faeces	..	21	All larvae hatched.
25	35° C.	Decoction of faeces	..	23	All larvae hatched.
		Faeces	Faeces	23	All larvae hatched.
26	35° C.	Decoction of faeces	Broken females	17	70 per cent. larvae hatched.

NOTE.—The terms first, second, and third lots were used to differentiate between eggs laid by the females after varying intervals from the time they were removed from the sheep and transferred to the artificial medium, thus :—

First lot indicates eggs collected in the first three hours after removal.

Second lot indicates eggs collected between the third and ninth hours after removal.

Third lot indicates eggs collected between the ninth and fifteenth hours after removal.

Conclusion.

1. From the above table it is evident that the interval after which eggs hatch varies in relation to the time that elapses between the removal of the female from the host, a shorter time being necessary for hatching eggs laid by the female three hours after removal, than in the case of eggs laid by females within three hours from removal.

2. Eggs obtained from faeces hatch sooner than eggs that are laid by the female. This is probably due to the fact that whilst the eggs pass through the intestines the evolution of the embryo continues. In cultures obtained from eggs in freshly-passed faeces, young larvae are soon visible under the optimal conditions of moisture and hatch 14-15 hours after evacuation. The majority of eggs hatch at the 19th hour; by the 22nd hour few eggs are found unhatched.

THE LARVAE.

Cultivation of Larvae.

Luekart obtained his cultures by placing pregnant females in water, whereupon the eggs escaped and the larvae developed. Parona and Grassi, Perroncito, Leichtenstern, Giles and Looss, used eggs obtained from evacuated faeces, using the latter as a medium, either pure or mixed with charcoal. Major Smith cultivated the eggs in distilled water, whilst Nissle and Wagner used a thin layer of agar on which eggs were spread out. In my experiments I used various methods, which varied with the object in view, viz. :—

- (1) Cultivation for diagnostic purposes; or
- (2) cultivation to obtain a stock of larvae for experiments; or
- (3) the cultivation of larvae to be studied in their free stage.

(1) *Diagnostic Purposes.*—When microscopical examination of the faeces is not resorted to, the presence of eggs can be determined by allowing them to hatch. This applies chiefly under conditions where the eggs are so rare that they may be overlooked even with a microscope. In such cases faeces were placed in a suitable beaker or dish, just covering the bottom, and were spread out with water when they dried up. The vessel was then covered to prevent evaporation and was either placed in an incubator at 26°-35° C., or kept at room temperature. If development occurred the larvae were seen to crawl up the walls of the vessel four days later, and could be recognized as white masses branching out in various directions. This method was found preferable to microscopical examination in cases where the infection was only a slight one. It allows of the easy detection of the embryo on the walls of the glass, and can be utilized by the layman to diagnose the state of infection in a flock of sheep.

In general examination the following procedure was adopted :—The animal under observation was placed in a box and the faeces were collected every morning. Sometimes they were obtained direct from the rectum by emptying it out with the finger, at other times a bag was placed against the anus and tied to the tail and the droppings thus collected. The faeces were then broken up and moistened. About 10 grammes of the mixture was placed in an ordinary test tube and, by means of a glass rod, distributed on the walls in two longitudinal rows so that the larvae could crawl over the intervening space. The necessary moisture for the hatching of the eggs was present in the faeces placed in the bottom of the tube. The tube was then stoppered with a cork or with cotton-wool plugs. A number of tubes were placed in a suitable receptacle, covered with a lid, and removed to the incubator.

(2) *Obtaining a Stock of Larvae.*—When the object was to obtain a large stock of larvae, consideration had to be given to the consistency of the faeces. Diarrhoeic faeces are a bad medium; they may be used if charcoal be added, although even then a proportion of failures must be expected. When material is collected from the caecum and colon on post-mortem examination, the cultivation of eggs is not always successful, probably owing to the presence of fermentation. The normal droppings of sheep are the best medium for the cultivation of larvae, and such were generally used.

After placing in a jar measuring 9 c.m. in diameter and 20 c.m. high, the jar was covered with a lid and kept at room temperature. Sufficient air was available inside the jar to permit of the eggs hatching, but when fermentation took place in the faeces all further development stopped.

As stated previously, the larvae appeared on the wall of the jar four days later and then crawled up to the top where they could be easily removed with a piece of blotting-paper. They were in a clean state, free from faeces. (It is necessary to have the vessel closed to prevent larvae escaping from the top.)

(3) *Studying the Larvae—Cultures made in Liquid Medium.*—It occurred to me that it would be advisable to devise means whereby a large number of larvae could be examined simultaneously in the medium, and whereby individual larvae could be removed for closer examination of their structure. This object was attained by using a transparent medium spread out in a dish and placed under the microscope.

The best results were noted by using a filtered decoction of faeces prepared in the following way:—

Two tablespoonfuls of normal faeces from a sheep were boiled in 100 c.c. of tap water; the mixture was then filtered, and the decoction poured into a Petri dish in a layer of about 1–3 mm. in thickness to which was added some eggs laid by the females. (The eggs developed if the layer of the medium was not thicker than indicated, otherwise failures were frequent.) The Petri dish was then kept in the incubator, and in order to prevent desiccation new medium was added from time to time. It was found advisable to place such Petri dishes in a covered evaporating dish, the bottom of which contained a layer of cotton-wool soaked in water. A modification of this method consists in using simply tap water as medium to which is added a small quantity of sheep faeces.

For the purpose of examination the Petri dish is placed under the binocular microscope, using ocular No. 2 and objective A2. In this way the hatching of the egg, or any developmental stages, can easily be observed and controlled on a number of larvae at the same time. By means of a pipette individual larvae can be picked out, transferred to a drop of water on a glass slide and covered with a cover-glass, when they can easily be examined under a higher power. The sudden change of temperature from the incubator to that of the room does not hurt the larvae. There seems to be no difference in the rate of development in larvae kept in liquid or in solid media. Sometimes it appears that solid cultures are more reliable than liquid ones. The fine granules in the body of the larvae seem

to be more numerous and of a larger size in solid media. The following table shows the difference in the development of the larvae in liquid and solid media :—

DATE OF CULTURE AND MEDIUM USED, NOVEMBER 11TH, 1913.	TIME OF OBSERVATION—NOVEMBER, 1913.					
	12th— 7 a.m.	12th— 10 a.m.	12th— 1 p.m.	14th— 11 a.m.	15th— 11 a.m.	16th— 11 a.m.
Stomach contents in water	60 percent. eggs hatched	99 percent. eggs hatched	5 per cent. larvae in first lethargus	25 percent. in second stage	50 percent. in second lethargus	Majority of larvae mature.
Stomach contents in earth	95 percent. eggs hatched	100 percent. eggs hatched	25 percent. larvae in first lethargus	50 percent. in second stage	50 percent. mature	Do.
Faeces in water	2 per cent. eggs hatched	40 percent. eggs hatched	100 per cent. eggs hatched	25 percent. in second stage	Do.	Do.
Moistened faeces	10 percent. eggs hatched	60 percent. eggs hatched	99 percent. eggs hatched	40 percent. in second stage	80 percent. mature	Do.

Cultures Made on Agar.—With slight alterations the agar cultures as suggested by Nissle and Wagner gave satisfactory results. These cultures were prepared and infected with eggs in a manner similar to that described under liquid cultures. It was found that a 0·5 per cent. agar concentration gave a better result than the 1 per cent. suggested by the above authors. Looss' criticisms concerning the unsuitability of agar as a medium (owing to bacterial contamination) were taken into consideration. The heated liquid agar was accordingly poured into the Petri dishes and immediately covered up. When kept at the ordinary temperature of the room they were found to be still useful after the lapse of a month. Agar plates inoculated with eggs obtained from females or from the faeces, and kept moist at 25°–30° C., were still suitable for examination fifteen days later. The growth of moulds at this temperature was quite insignificant and did not interfere with the object in view, always provided that the Petri dishes were kept covered. As an extreme case in point, it may be mentioned that agar plates inoculated on 5th March, 1915, were still suitable for microscopic examination on 27th June, and still provided larvae for further investigation.

It is interesting to note that cultures in faeces with charcoal, kept at a temperature of 40° C., failed, but succeeded when made with agar and faeces. At high temperatures the agar cultures gave better results. On the other hand, at a low temperature the agar cultures were the first to fail. The reason for this may perhaps be that at high temperatures decomposition occurs in the faeces to a greater extent than in agar culture, even if the faeces are mixed with charcoal.

Method of Culture used by Major F. Smith.—Liquid cultures, according to the method of Major Smith, are made by placing eggs in pure water. A number of cultures were made on these lines and the result of the observations on one of them is given hereunder.

The females were collected from the stomach of a freshly-killed sheep, were broken up, and the eggs separated from the fragments; the cultures with the eggs were inoculated in the following media:—

- (1) Decoction of faeces (control culture).
- (2) Pure water.
- (3) Water containing detritus of the broken-up females.

Twenty-four Hours Later.

The larvae in all cultures were in the first stage.

Forty-eight Hours Later.

Culture 1.—The larvae were in the second stage.

Culture 2.—The larvae were in the first stage, and poor.

Culture 3.—Few larvae were in the second stage.

Seventy-two Hours Later.

Culture 1.—The larvae were in advanced second stage.

Culture 2.—Numerous larvae had died; the remainder were in the first stage.

Culture 3.—Numerous larvae had died; the remainder were in the first stage.

One Hundred and Twenty Hours Later.

Culture 1.—The larvae were all mature.

Culture 2.—All larvae had died in the first stage.

Culture 3.—90 per cent. were dead; the remainder were poor.

One Hundred and Forty-four Hours Later.

Culture 3.—The remaining 10 per cent. had died.

Conclusion.—Eggs will hatch in pure water, but the larvae do not develop and soon die of starvation. In cultures containing detritus of the mothers, the larvae reach a later stage, but finally die, probably owing both to putrefaction of the medium and to starvation.

DEVELOPMENT OF THE LARVAE.

In their evolutive cycle the larvae of *Haemonchus contortus* pass through five stages separated by structural changes. The two first stages and part of the third one are passed outside the host, as free larvae. The second part of the third stage, the fourth and fifth stages, are passed inside the host as parasites. Each stage can be divided into two sub-stages: the first one in which the larvae move rather actively, feed, and develop; and a second one in which the larvae are found in a lethargic condition, in which they neither move nor feed, but undergo structural change. Entering the fifth stage the worm is sexually mature and is usually called the *Adult Worm*.

First Stage.

Size of the Larvae.—The naturally hatched larvae vary in size from 340–350 μ in length and from 15–20 μ in thickness, the average being 345 μ \times 17 μ respectively (the thickness measured at the base of the œsophagus). Morphologically, the larvae belong to the rhabditoid type. The form of the body is cylindrical, decreasing in thickness from the base of the œsophagus to the tail. This form is typical of the first stage and an accustomed eye can at once distinguish it from the second stage.

Skin: [NOTE.—The longitudinal pad-like formations of the cuticle projecting externally over the body will be called *longitudinal lines*. The similar formations of the sub-cuticle projecting in the coelomic cavity will be called *longitudinal bands* (Fig. 21A). These terms will be used both for the larvae and for the adult worm.]

The skin shows very slight transverse striations. On each side of the body a very fine, sometimes dark and sometimes more transparent “longitudinal line,” with a sharp edge, can be discerned, starting some distance from the head and ending a little behind the anus (Fig. 16). Below this lateral line the lateral band can be detected of 6 μ in width. The dorsal and ventral bands can only clearly be recognized in cross sections as a simple deviation between the two quadrants of muscles.

Digestive Apparatus.—The mouth aperture is circular and slightly conical, ending suddenly in the buccal cavity. On the edge of the mouth opening can be seen six transparent dots about 3 μ in diameter. The external diameter of the mouth aperture is 7 μ .

The Buccal Cavity.—The buccal cavity is 14 μ in length—excluding the mouth opening, measuring 3 μ . Under the microscope the walls appear as two sharp refrangent lines, ending posteriorly in two dots (Fig. 15).

The Œsophagus.—The œsophagus is rhabditoid in shape, and the constriction slowly passes over into the bulb.

The lumen appears as a clear line, and the “Y-shaped mark” in the centre of the bulb is quite clear. The walls of the œsophagus are thick, transparent and finely transversely striated. The length of the anterior portion is 42 μ with a thickness of 9–10 μ ; the constricted portion is 28 μ long and 5 μ thick, and the bulb is 14 μ long with a diameter of 13–14 μ (Fig. 15).

Chyle Intestine.—This extends throughout the body from the œsophagus to the rectum. When observed laterally, the lumen has a zigzag course, slightly funnel-shaped at its anterior opening. The wall consists, dorsally and ventrally, of one row of eight cells; they are roughly triangular in shape. The average length of the cells is 18–20 μ \times 9–10 μ in thickness. The nucleus is large, round or slightly oval. The protoplasm contains fine granulations, increasing in number with the growth of the larvae. The two rows of cells are arranged in an alternating order, the protruding part of one row being opposite the marginal parts of the opposite ones. The connection between the chyle intestine and the œsophagus is effected by two rings, which on longitudinal section appear composed of two cells, each containing a nucleus.

The Rectum.—The rectum is 12μ long and runs in a slanting direction, passing through a thick granular mass. The anus lies on the ventral side of the body, the edges slightly protruding. The last part of the rectum has a chitinous lining.

Nervous System.—In specimens stained with borax or hydrochloric acid carmine, or in larvae that have been starved in order to cause the granules to disappear, it is possible to recognize the outlines of the nervous system. At the constriction of the œsophagus the nerve ring appears as a band surrounding the œsophageal wall. The ring is surrounded by a number of cells, of which the majority belong to the nervous system. The cells are arranged in lateral and post-lateral groups, the former lying just behind the nerve ring and extending backwards (ganglion cephalicum laterale); another group lies ventrally (ganglion cephalicum ventrale). In the neighbourhood of the rectum a group represents the primordium of the anal ganglion.

Excretory Apparatus.—This is represented by a short canal opening on the ventral side, situated in front of the œsophageal bulb and entering into a group of cells found in that region.

Genital Primordium.—This can be recognized as a small elongated body, 12μ in length. It apparently consists of two cells. It is placed ventrally between the chyle intestine and the body wall, at a distance of about 25μ in front of the anus.

Biology of the First Stage.—As stated previously, two sub-stages can be recognized, one of activity and one of lethargy. The period of activity is noted subsequent to hatching of the eggs. The young larvae move actively in a series of wriggling movements followed by short periods of rest. After about an hour steadier movements are noted and the larvae commence to feed. In larvae at this stage a contraction of the intestine can be noticed, causing a bulging of the posterior end. The larvae do not travel any distance in water. The movements are somewhat slow and very typical. They serve to distinguish the first from the second stage. Later, when the cells of the chyle intestine are packed with granules, the larvae stop feeding and attempt to enter into the medium. In plate cultures the majority of the larvae entered the agar when their movements slackened down. Finally they became stationary. This represents the beginning of the lethargic stage. Similar observations can be made in larvae kept under natural conditions. This lethargic stage apparently has to be undergone in moist surroundings protected from the direct action of the sunlight. On examining pellets of droppings which have partially dried in the open, only dead larvae and eggs can be detected on the surface; living larvae are found within or between the faeces, and on the ground on which they are placed. Of these larvae some belong to the second stage, others are still in the first stage and are either at, or near, the first ecdysis. Apparently the newly-hatched larvae, whilst feeding, are attracted towards the superficial layers of the pellet where they pass a period of activity. Afterwards the larvae penetrate into the interior of the faeces, where they undergo the first lethargus, waking up again as soon as structural changes have occurred.

First Lethargus.—The length of the active period varies according to the condition of the culture. In liquid cultures, I found that after eight hours a good number of larvae had reached the first lethargus, and after ten to twelve hours the majority were motionless. This corresponds to twenty-four to twenty-seven hours after the eggs have been passed out by the host. The larvae take a comma-like shape and are rigid. If the liquid culture is shaken or exposed to a bright light for some minutes, the larvae do not react. This fact is so remarkable that when first noticed I thought the larvae were dead, but when the culture was examined later some larvae were found to be in a more advanced stage and further developed. This lethargic stage cannot be detected in a solid medium, because when the larvae are removed and transferred to a liquid medium for the purpose of examination they revive. On account of the unequal development of the larvae in faeces, the larvae of the two sub-stages (activity and lethargus) are usually mixed together, and the appearance of the lethargic stage is not so striking. With the beginning of the lethargus the larvae appear shorter. It can frequently be noted that the constricted part of the oesophagus bulges out in the form of a loop (Fig. 17), the intestinal lumen having lost its regular zigzag shape. The length is now reduced to 350μ as a minimum; in most larvae it averages $400\text{--}450\mu$. It is during this lethargic stage that a separation takes place between the old and the new skin (Fig. 17). This is preceded by a thickening of the old skin, under which the striation of the new one makes its appearance. When more advanced, a new tail appears within the skin of the old one. At the same time the internal structure undergoes slight modifications, the chief ones being a division of the nuclei in the chyle intestines and the appearance of the lateral lines of the new skin, slightly raised, and with sharp edges. After eight or ten hours when the larvae revive, all these structures are more easily recognizable. It is possible that the surrounding moisture—or rather the penetration of the moisture through the skin into the larvae—has something to do with the awakening. There exists some difference of opinion as to when the second larval stage commences. Previous authors describe the second stage as beginning after the larva has undergone its moulting. From the biological point of view larvae are already in the second stage when the structural changes mentioned above have taken place, that is, from the moment that the larvae awaken again. Casting of the skin is purely mechanical and coincides with the activity of the larvae at the beginning of the second stage. The moulting process is independent of the further development of the larvae. Directly the anterior end of the outer skin has been thrown off the larvae begin to feed, even if the remainder of the skin has not been stripped. Consequently, the first ecdysis will be included in the description of the second stage.

Second Stage.

First Ecdysis.—The first act of the awakening larva is to get rid of the old skin. The mechanism of the first ecdysis was frequently observed in a liquid medium, and the following notes were made:—

When the larvae are first awakened the crawling movements are very prevalent. The larvae seem to prefer to pierce into pieces of faeces that

offer resistance. At this time the old tail is bent in the form of a hook, and the larva is firmly attached by means of this hook to a piece of faeces, as it begins to free itself from the skin (Fig. 18). It can also be noted that when the larva forces itself through particles of the faeces, the anterior part of the skin separates and remains hinged to the old skin in the form of a hood (Fig. 18). The hood is also occasionally separated completely, as if pushed away, probably owing to a sudden increase of pressure inside the skin. This ecdysis is probably also connected somehow with this pressure. In the liquid medium the cast skins are frequently found floating on the surface, sometimes with the hood, which is about 15μ in length, still attached. The skin is frequently well preserved, the lateral line being quite distinct, as well as the transverse striation and the cephalic papillae.

The Period of Activity of the Second Stage.—A marked growth seems to take place soon after ecdysis, as the length of the larvae is now found to reach 500μ . All the new structures can be observed; the lateral lines with a width of 3μ are conspicuous and sharply set off (Fig. 20—Fig. 21A *l. lat.*). The stage of the worm is thus characterized by the structure of the lateral lines. The larvae are cylindrical in shape and of equal diameter from the bulbus of the oesophagus to the anus. The activity in this stage shows itself in swimming movements. The larvae begin to feed again and the intestinal lumen, which previously had a zigzag appearance, now seems to straighten out and increase in diameter, whilst the cells of the intestinal wall become more flattened. The granules in this wall increase and the cells return again to normal shape. Once the larvae have obtained sufficient food they can be noted to move again, with the idea of hiding in the interior of the medium. A peculiar feature is that in liquid media, the larvae are found at the bottom of the liquid and apparently make no attempt to rise to the surface, whereas once maturity is reached they will do so.

Second Lethargus.—The moving larvae gradually become motionless and assume a bent or stretched appearance. The onset of the second lethargic stage depends either on external influence or individuality, a fact which is borne out in the following table:—

No. of Experiment.	Temperature.	Medium.	Eggs Obtained from.	Time after Hatching.	REMARKS.
19a	35° C.	Decoction of faeces	Laid by females	3 days	50 per cent. in second lethargus.
19b	35° C.	"	"	3 days	70 per cent. in second lethargus. (In some larvae second skin noticeable.)
9	35° C.	"	"	3 days	90 per cent. in second lethargus; some mature.
12	35° C.	"	"	3 days	50 per cent. in second lethargus; a few mature.
20	35° C.	"	"	3½ days	80 per cent. in second lethargus; many mature.
24	30° C.	"	Stomach contents	2½ days	50 per cent. in second lethargus.
25	30° C.	"	"	2½ days	90 per cent. in second lethargus.
		Faeces	"	2 days	90 per cent. in second lethargus.
		"	Faeces	2½ days	90 per cent. in second lethargus.

From the above table it can be seen that on the 3rd day a large number of larvae are found in the second lethargus. As a result of a number of observations, I came to the conclusion that the beginning of the second lethargus takes place about forty hours after the first ecdysis, or sixty to sixty-five hours after hatching. The size of the larvae at this stage varies, and this variation in size is connected somewhat with the medium in which they grow. The following table brings this fact out clearly:—

Experiment.	Eggs Obtained from.	Medium.	Time in Hours.	Size. μ	Condition of Capsule.
11	Laid in incubator at 30–35° C.	Decoction of faeces	68	600 \times 26.42	Outer skin evident.
15	"	Faeces	65	651 \times 24	Outer skin not apparent (œsoph. rhabditoid).
23	Faeces	"	64	691 \times 23.31	Outer skin not apparent.
23a	Stomach contents	Decoction	72	599.80 \times 23	Outer skin evident.
24	"	"	70	682.65 \times 23.31	Outer skin not evident (œsoph. rhabditoid).
24	"	Faeces	70	632.70 \times 23.31	Outer skin evident.
28	Faeces	"	65	756 \times 28	Outer skin evident (œsoph. rhabditoid).
29	"	"	65	754 \times 28	" "
30	"	"	65	756 \times 28	" "
31	Stomach contents	Stomach contents and charcoal	72	715 \times 27.5	" "

The structural changes which take place during the second lethargus are more pronounced and bring the larvae to the mature stage. These structural changes take place in the following order:—

- (1) The mouth aperture loses its conical shape.
- (2) The buccal cavity is narrower, and apparently there is no longer any distinction in the entrance to the œsophagus.
- (3) The constriction of the œsophagus becomes less marked or the œsophagus itself loses its rhabditoid form, and, as already mentioned in the first lethargus, is frequently found in the form of a loop.
- (4) The œsophageal valves are distinct, the granules of the chyle intestines become scarcer and more transparent; the lumen is more distinct.
- (5) The skin appears thicker and the body is slightly reduced in size.

The final appearance may be described from the observations made on a particular larva, of which the body measured 715μ with a diameter of 27.5μ , the tail length being 130μ . The outer skin was refragent and separated from the body, but still adherent with the exception of the head, where for some microns in length the skin was detached. The mouth opening of the outer skin was closed. The striations of the inner skin were well marked. The oral opening of the encysted larva was open. The mouth cavity was reduced to a simple line. The oesophagus was 175μ in length and 120μ in diameter at the base. The three portions of the oesophagus were hardly distinguishable and the whole organ appeared to be claviform in shape, with the posterior end as the base. The intestinal valves were not distinct. The cells of the chyle intestine were sixteen in number, roughly triangular in shape, measuring 50μ at the base and 20μ in thickness; these cells were finely granulated, showing a nucleus in the centre measuring 2μ in diameter. The anus was closed, but still adherent to the corresponding mark in the old skin. The genital primordium was situated at a distance of 175μ from the anus, measuring 20μ in length with a diameter of 7.5μ .

The nervous system is more developed, but appears confused with the surrounding cells.

Biology of the Second Lethargus.—After about twelve hours the development of the lethargic stage is completed, although under optimal conditions a period of even eight hours may be sufficient. With the accomplishment of the structural changes the larvae awaken, but if external conditions are unfavourable awakening is retarded. This lethargic state evidently serves not only for further development, but also represents a latent state for the larvae under unfavourable conditions. Here again the end of the second stage may be recognized when the larvae have undergone structural changes. The succeeding ecdysis would therefore be part of the third stage.

Third Stage.

Mature Larvae.—The larvae awaken from the second lethargic stage as soon as conditions are favourable; in general, three days after hatching. A low temperature and dryness delay awakening. The awakening is succeeded by the coiling up movements, and as a result of this, separation of the inner newly-formed skin takes place from the older one. This detachment shows itself distinctly by the difference in the situation of the two corresponding openings of the anus. Once this detachment has taken place, the larvae seem to have acquired the necessary freedom to enable them to travel about. Their movements are now fast as compared to those noted in the second stage, and they can best be described as "swimming." Another peculiarity is the attempt of the larvae to rise. These two phenomena are typical for larvae in the third stage. Once the larvae have reached maturity, their next object is to reach a suitable locality from which they can find access to the host.

Description of the Mature Larva.

Length of the Larvae.—The following table shows the various lengths of larvae grown in different cultures and for varying lengths of time:—

Experi- ment.	Eggs Obtained from.	Medium.	Time.	Size. μ
1	Laid by females	Decoction of faeces at 35° C.	20 days	571 × 21
2	"	" " "	20 days	630 × 22
3	"	" " "	45 days	614 × 23
3	Stomach	" " "	10 days	682 × 23·31
4	Faeces	Faeces	—	680 × 23
5	Stomach	Decoction of faeces at 36° C.	3 days	685·95 × 23·31
6	Faeces	Faeces	38 days	614·20 × 25·5
				632 × 25·2
				620 × 25·2
7	"	Faeces in the open at 30° C.	2 months	714 × 26·30
8	"	" " "	2 months	784 × 23·80
9	"	Faeces in the open at 35° C.	1 month	799 × 26·5
10	"	Faeces in the open at 32° C.	10 days	820 × 26·5

Shape.—In shape they do not differ from that described in the second lethargus (Fig. 21).

Old Skin.—The mouth opening of the outer skin is closed and a small appendix represents the old wall of the mouth. The marks of the six head papillae are distinct, as well as remnants of the rectal walls. The lateral lines are conspicuous, having the base wedged into the cuticle (Fig. 21A, *l. lat.*); the transverse striations are visible.

New Skin.—The new skin is also ringed. The distance between each ring measures $1\cdot7\mu$. The lateral lines have a width of $3\cdot4\mu$ and project from the circumference of the body for $1\cdot5\mu$, with the margin slightly protruding laterally (Fig. 21A).

Subcuticle: Lateral Bands.—In the whole specimen only the lateral bands can be distinguished. They appear at two sides of the body as two longitudinal strands, about 4μ broad. It is rather easy to distinguish these two strands, because they are more transparent than the two neighbouring muscular quadrants. On cross section the lateral bands appear finely granular and protrude into the coelomic cavity (Fig. 21A, *b. lat.*). The dorsal and ventral bands appear in cross section as a small granular triangle, wedged between the two neighbouring muscular sectors (Fig. 21A, *b. ven.* and *b. dors.*).

Musculature.—The muscular arrangements are divided into four quadrants, two dorsally and two ventrally. The muscle cells are rhomboidal, measuring about 45μ in length. Their longitudinal axis runs parallel to the longitudinal axis of the body. On cross section each muscular quadrant occupies 11μ of the circumference of the body and protrudes for a distance of $5\cdot5\mu$ into the cavity. Each quadrant is composed of seven muscle cells (Fig. 21A-M).

Mouth.—The mouth is closed. The mouth cavity starts with a small globular dilatation, and extends as a fine canal into the œsophageal lumen.

The Œsophagus.—The œsophagus is more or less claviform in shape, but has a slight constriction that can only be detected under a high magnification (Fig. 21). The lumen of the œsophagus appears as a straight stretched line with a slight dilatation at the posterior end, where the œsophageal valves are present as described in the previous stage. In the substance of the walls numerous fine granules are seen arranged in longitudinal strands, representing the primordium of the œsophageal glands. The intestinal valves consist of the two rings described previously. The chyle intestine is composed of sixteen cells, more or less rich in granules according to the state of preservation of the larvae (Fig. 21A, int.). The lumen still retains the zigzag appearance described previously.

Nervous System.—It is difficult to examine the nervous system in living or unstained larvae, but staining with aqueous methylene blue renders examination possible, the nervous system being more conspicuous than other organs. Generally speaking, the nervous system corresponds to what has been described previously, although the outlines are now more distinct. The cerebral commissure appears as a band situated $75-80\mu$ from the anterior end. It is finely striated and does not show any nuclei. In breadth it measures 5μ . Anteriorly, some longitudinal strands of nuclei surround the first part of the œsophagus, representing the primordium of the papillary nerves. Posteriorly to the nerve ring the two lateral cephalic ganglia stain a deep and distinct blue and appear as two compact masses of nuclei, totalling 30μ in length. The ventral ganglion is represented by a group of nuclei, not so compact as the two just referred to, and extends from the cerebral commissure to the end of the œsophagus and surrounds the excretory canal.

In the whole specimen, the nerves running along the longitudinal bands and connecting the central system with the posterial ganglion, were not sufficiently distinct for identification. In cross section three dots in the coelomic cavity were seen (Fig. 21A), and apparently correspond to the two lateral and the ventral longitudinal nerves.

The genital primordium is represented by about sixteen nuclei, taking the methylene blue stain well, and occupying an oval area of about $9.5\mu \times 8\mu$, situated obliquely to the medial axis in the ventral region and about 320μ distant from the tip of the tail.

The excretory canal leads backwards into a mass of nuclei, which represents the primordium of the excretory organ, but which cannot be completely distinguished from the nuclei of the lateral and ventral ganglia. The aperture is on the ventral side, about 80μ from the head.

Larvae under Natural Conditions.—In order to control the biological facts noted from liquid cultures, a series of observations was made in the open in the following manner:—

Faeces from infected sheep were placed around a tuft of grass in a field. The faeces were moistened with water and covered with a glass bell in order to conserve the moisture. The temperature varied between 35°C . in the day time and $12-15^{\circ}\text{C}$. at night time. On the 1st day the larvae were found in the first stage and more or less uniformly distributed

throughout the droppings. On the 2nd day the majority of the larvae were in the second stage and were found both in the lower parts of the pellets of faeces or in between the pellets themselves, evidently sheltering from sunlight and trying to obtain the maximum moisture. On the outside of the pellets, unhatched eggs, or dead larvae in the first stage, were found; inside and in between the mass of pellets, larvae in the first stage and in the first lethargus were most frequent. On the 3rd day a very few larvae in the first stage were found in the uppermost pellets, whilst in the pellets touching the ground large numbers of larvae in the second stage were found. During the first three days no young larvae were found crawling up the grass. On the 4th day 80 per cent. of the larvae were found in the lower layers of pellets, the remaining 20 per cent. being on the upper layers. The first of the mature larvae were now found crawling on to the grass. The transition from the lethargic to the mature stage is, biologically, not so distinct under natural conditions as it is in liquid cultures, where it is detected by activity of the larvae, but nevertheless mature larvae can easily be recognized by their morphology. In the course of the following days numbers of larvae were still found to have undergone development and to have reached maturity, whilst a continuous migration could be observed on the grass. During the next seven to fifteen days a few larvae were still observed in the different layers of the faeces, representing the various stages of growth from hatching to maturity. Underneath the pellets and in contact with the ground, a number of larvae in the second lethargic stage were found.

Further Changes of the Mature Larvae.—When larvae have reached the mature stage, a certain contraction of the tissues takes place by which the size of the body sensibly decreases, whilst the outer skin becomes thicker and increases in rigidity. As long as larvae were kept in a moist ambient, and other conditions for their preservation were favourable, the body filled the outer skin, but when unfavourable conditions intervened, the outer skin contracted, with the result that the space in between the skin of the body became distinct (Fig. 22). The second feature found in larvae in the mature stage is the presence of vacuoles in varying numbers, and of different sizes, in the chyle intestinal cells. These vacuoles are frequent in living larvae that have been preserved, or in larvae exposed to unfavourable conditions, such as dryness, light, or heat, and the presence of numbers of them indicates that the larvae are nearing death (Fig. 22-23).

The third observation in the mature larvae was the completion of the second ecdysis. This phenomenon occurred at different times, according to the medium used, and the following observations can be recorded:—

(1) *Hydrochloric Acid Solution, 1-5 per cent.*—A few larvae freed themselves of the outer skin shortly before death. The observation with 1 per cent. solution was continued for three days, by which time 60 per cent. of larvae were dead. The observation with 5 per cent. solution was continued for twenty-four hours, by which time all the larvae were dead.

(2) *Concentrated Aqueous Solution of Methylene Blue or Trypan Blue.*—Completion of the second ecdysis had not occurred after the larvae had been soaked in the solution for some days.

(3) *Gelatine Solution*.—Dry gelatine was soaked in water for a night. The superfluous water was then removed and the gelatine was liquified in a water bath. The gelatine was then allowed to get cold, and when at a temperature of 30–40° C., it was poured into a Petri dish where mature larvae had been placed in a few drops of water. The temperature of the room was 15° C. Next day the gelatine layer was firm. All larvae still retained the outer skin. Those on the surface were moving, whilst those in the mass of the gelatine were motionless. The Petri dish was then kept at 25° C. Two days later the larvae were partly in the gelatine and partly coiled up on the walls of the Petri dish. Practically all of them still had the outer skin.

In a second experiment a 1 per cent. solution of gelatine was made. In some dishes, cotton-wool flakes were added, whilst in others glass filaments were mixed with the gelatine and distributed over the surface.

Three days later the preparations were examined and all larvae had their outer skins intact.

Of larvae kept in ordinary tap water in a Petri dish at room temperature and in a weak diffused light, some were found dead without the outer skin after four to five months of preservation, whilst the remainder were living and ensheathed in the outer skin.

In a parallel observation mature larvae were placed in water 20 c.m. deep and kept under conditions similar to the above. Two and a half months later 90 per cent. of larvae were without their outer skins, and of these only 2–3 per cent. were dead. Three and a half months later very few larvae were found with the outer skin; 80 per cent. of the larvae which had completed the second ecdysis were living.

On the walls of the glass in jar cultures, dead larvae were frequently found without their outer skins, from the sixth or seventh months of observation. I am inclined to consider the casting of the skin in such cases as a sign of approaching death. There are numerous other cases, however, in which it would appear that mechanical injury or friction is mainly responsible for the second ecdysis. For example, larvae escaping through crevices in the lids of culture jars are in most cases found with their outer skins wholly or partly cast. This effect of mechanical friction is also suggested by certain experiments carried out in connection with the mode of infection of the host. The experiments themselves are discussed more fully in a later section, but at this point it may be incidentally observed that larvae placed upon the shaved skin of sheep and protected by a bandage, rapidly lost their outer envelopes; also that after subcutaneous injection of an aqueous suspension of larvae the majority were found without their outer skins, but still alive, when examined eight days later.

No explanation can be given for the fact that the larvae kept in water remained alive for several months without their outer skin.

INFLUENCE OF THE AMBIENT ON EGGS AND LARVAE.

In discussing the influence of the ambient on the free life of *Haemonchus contortus*, eggs and larvae will be treated together.

Intoxication of Eggs and Larvae.

The first factor interfering with the evolution of the egg, and even sometimes effecting its destruction, is the toxic substances of the intestine of the host. The previous remarks concerning the use of faeces as a medium for culture apply here as well. The fact that toxic substances may be found in evacuated faeces as a result of abnormal fermentation was mentioned when considering the cultivation of larvae. In the normal course of events the shells of the eggs as well as the cuticle of the larva are impervious to the surrounding liquid. It therefore appears that the toxic substances influence this protective property, rendering the membranes permeable, and thus intoxicating the egg-cells or the larvae.

Poisoning of Eggs and Larvae.

The action of certain chemical compounds upon eggs and upon larvae was investigated. Beta-naphthol, thymol, picric acid, and copper sulphate were each tested upon liquid cultures of eggs at the morula stage. After four days at 25–30° C. all the treated eggs were opaque and dead, while those in control cultures developed to mature larvae. The compounds mentioned not only inhibit development, but actually destroy the vitality of the egg. A number of other compounds were also tried and it was found that, in general, those which were injurious to the egg were also injurious to the larvae. This point will be taken up again in considering the medical treatment of infected sheep.

Air.

Eggs and larvae of *Haemonchus contortus* require a certain amount of air for development. The amount needed is very small, but if it falls below a certain minimum, inhibition of growth, and finally death, will occur. As indicating the necessity for air is the fact that cultures of faeces do not develop if made into too thick a paste with water, or if carried out in liquid media more than 3 mm. in depth. The difference in development between larvae found on the surface of pellets of sheep-dung, and those found in the interior, may also be explained upon differences in the oxygen supply. In a subsequent section dealing with the effect of moisture upon eggs and larvae, detailed experiments will be given which illustrate the influence of aeration as affected by the depth of aqueous layer in which the eggs are placed.

That the oxygen requirements are low, however, is suggested by the success of the method already described as used in routine cultivation for diagnostic purposes. The tubes containing the faeces were corked up and stored in a closed jar, and free access of air was therefore limited. Nevertheless the development of the cultures was always satisfactory.

Certain other experiments, carried out on the lines adopted by Looss in his work on *Ankylostoma Duodenale*, point in the same direction. Infected faeces, with eggs in the morula stage, were collected in small specimen tubes 3 cm. high by 1.5 cm. in diameter. The amount of faeces taken was 0.5 to 1 c.c., mixed or dusted with charcoal, and the tubes were hermetically sealed with wax. After from ten days to one month's storage at 24° C., numerous mature living larvae were found coiled up on the walls of the tube. A number of dead eggs in the morula stage were also found.

I am of opinion that more air is required for developing eggs and larvae than for resting eggs and mature larvae. Mature larvae kept in water at a depth of 3 cm. were still alive at the end of five months. Kept at a depth of 20 cm., 50 per cent. were still found alive at the end of three months. Larvae which attempted to escape from culture jars were found alive four months later in the vaseline jelly used for sealing.

Temperature.

With regard to the action of temperature on growing and maturing larvae, some experiments were carried out under laboratory conditions, and as controls another series was undertaken under natural conditions of the veld. When working with high temperatures special consideration was given to decomposition of the culture media, which had a lethal effect more pronounced with increase of temperature. Consequently, as a medium consisting of faeces alone easily undergoes fermentation, I frequently took recourse to a mixture of faeces and charcoal. In other cases liquid nutrient medium or plain water was used, and later agar plates were utilized. The latter proved the most satisfactory. It may be stated at the outset, that the optimum temperature for the development of eggs and larvae was found to be between 20° C. and 35° C. Accordingly, I call *normal temperature* that lying between 20° and 35° C., *high temperature* that above 35° C, and *low temperature* that below 20° C.

High temperatures acting for a short time.—A number of mature larvae of *Haemonchus contortus* were kept for microscopical examination in glass Petri dishes of 10 c.c. capacity. The various dishes were filled suddenly with water of varying temperature and then left at laboratory temperature of 20° C. The results are given in the following table :—

TEMPERATURE OF THE WATER.	EFFECT OF WARM WATER ON THE LARVAE.		
	Immediate.	A Minute Later.	24 Hours Later.
50° and 53° C.	Coiled up or curved	Nearly all moving	Very few dead.
55° C.	" "	Some not moving.	Very few dead.
58° C.	" "	90 per cent. moving	2-3 per cent. dead.
60° C.	" "	70 per cent. moving	3-4 per cent. dead.
63° C.	" "	60 per cent. moving	4 per cent. dead.
65° C.	" "	50 per cent. moving	10 per cent. dead.
	(Few distended)		
68° C.	" "	8-10 per cent. moving	10 per cent. alive.
70° C.	Distended	8-10 per cent. moving	5 per cent. alive.
73° C.	" "	2-3 per cent. moving	2-3 per cent. alive.
75° C.	" "	All distended	2-3 per cent. alive.
78° and 80° C.	" "	" "	1-2 per cent. alive.
85° and 90° C.	" "	" "	All distended, and dead.

Conclusion.—At temperatures between "normal" and 65° C. a very small proportion of larvae were killed, whereas at temperatures between 65-70° C. the majority survived. A temperature of from 70-80° C. is decidedly fatal to the majority of the larvae and very few survive. At 85° C. no live larvae were found. Practically, one may say that immersion in water at a temperature of 85-90° C. kills all the larvae instantaneously.

Eggs and larvae exposed to high temperatures : 60° C.—From a fifteen days old culture made in faeces, in which practically all the larvae were alive, a sample of the medium containing a good number of larvae was placed in an oven at 60° C. At the same time larvae were fished from the walls of the jar of the same culture, immersed in water and also placed at 60° C.

Periodic examination under the microscope of both batches gave the following results :—

Larvae exposed for thirty minutes in faeces appeared motionless and stretched out. After one to two minutes of observation 90 per cent. awakened and were swimming.

Exposed for an hour in faeces.—After one to two minutes of observation 30 per cent. awakened and were swimming.

Exposed for two hours in faeces.—After ten minutes of observation some larvae were observed to coil up, occasionally showing movements, the remainder were dead.

Two and a half hours of exposure.—All the larvae kept in water or faeces medium were stretched out and dead.

This experiment was repeated several times, with much the same results. When the temperature was not disturbed by intermittent opening of the incubator, two hours' exposure usually sufficed to kill all the larvae.

Cultures of Eggs and Mature Larvae kept at 50° C.—Eggs in the morula stage kept in faeces or on agar media for six hours were killed in the morula or tadpole stage. Kept in liquid media, eggs in the last stage were found to have hatched but the larvae were already dead. Mature larvae in faeces or in agar were found dead and stretched out. Attached to a piece of dry grass a few were found alive.

In reducing the time of exposure to four hours eggs were found dead ; the mature larvae were found dead if exposed in liquid medium, but some were still found alive after exposure on dry grass.

Eggs in the morula stage kept for two hours on agar were sometimes found dead, but it was nevertheless not rare to find the eggs still alive. Out of five attempts two gave positive results. The surviving eggs put up for culture reached maturity. Mature larvae kept for two hours in liquid media were usually found alive, very few deaths having occurred. Eggs at the morula stage kept in agar and exposed at 50° C. for two hours daily, and in the intervals kept at 28° C., hatched in two out of eight attempts. The larvae reached the second stage on the second day. After the third exposure the larvae were placed constantly at 28° C. On the seventh day all were dead without having reached the mature stage.

Mature larvae in agar plates were also exposed to 50° C. for two hours daily. After the third exposure 20-30 per cent. were found to have been killed. In the further course of this experiment it was not possible to establish the percentage of deaths, owing to the living larvae crawling on the walls of the dish and escaping, but I was able to find living larvae after the sixth exposure.

In connection with these notes concerning the resistance of larvae at 50° C., the following further experiments are also of interest:—

Some pieces of dry grass, on which were numbers of larvae twelve days old, were kept in an incubator at 50° C. and were divided into different batches. After pre-arranged time of exposure each lot was transferred to water at normal temperature and examined twenty-four hours later. The result was as follows:—After an exposure of—

2	hours,	2	per cent.	of the larvae	were alive	and swimming.
4	„	6	„	„	„	„
6	„	1	„	„	„	„

After twelve and twenty-four hours all the larvae were dead.

The apparent discrepancy in the results can be explained in the light of the ascertained distribution of the larvae on the grass. Microscopic examination of the material before placing in the incubator had revealed the presence of numerous larvae uniformly distributed on the grass blades, and of some clusters of larvae measuring about 100 μ in thickness. The small percentage of surviving larvae after two hours' exposure probably occurred amongst those which were uniformly distributed on the blades; the 6 per cent. of surviving larvae after four hours' exposure and the 1 per cent. after six hours' exposure is probably represented by larvae collected in the centre of the clusters, which were thus well protected from the direct action of heat and evaporation. This experiment also throws some light on the striking differences obtained in various experiments as recorded by other authors. We are justified in concluding that larvae kept at a temperature of 50° C. in a dry incubator do not resist longer than two hours if well exposed to the ambient, whilst those present in the centre of clusters can resist for six hours. In the veld, larvae are probably well sheltered and thus able to resist high temperatures. This point was also investigated experimentally on the following lines:—

Mature larvae were collected on blotting paper and placed in the centre of pieces of dry black earth. One piece measured 3 cm. in diameter, and the other 6 cm. The two pieces were then kept in a dry incubator at a temperature of 50° C. The first one was examined twenty-four hours later, when 5 per cent. of the larvae were still alive. These results give a good idea of the resistance shown by larvae when sheltered, even if exposed to the high temperatures found in the summer months.

45° C. —Cultivation of eggs in the morula stage was attempted in faeces on three occasions, and in charcoal-faeces on five occasions, but in each case with negative results. Eggs at the morula or tadpole stage were also maintained at 45° C. in agar cultures for three or four days and then removed to 38° C., but all failed to develop. Daily exposure of eggs in charcoal-faeces or in agar, for six hours on three successive days, also resulted in the death of the eggs. In the same media, however, eggs were found to withstand a daily exposure of two hours for four days. In charcoal 50 per cent., and in agar 80 per cent., of mature living larvae were found.

Mature Larvae at 45° C.—The various observations can be summarized and are given in the following table:—

Time of Exposure.	In Pure Water.	In Agar Cultures.	In Cultures of Faeces.	In Cultures of Faeces plus Charcoal.	On Dry Grass.
5 hours.....	90 % alive	—	—	—	—
8 „	—	80 % alive	—	—	—
12 „	50 % alive	—	50 % alive	60 % alive	—
18 „	—	70 % alive	—	—	—
24 „	20 % alive	60 % „	—	—	10 % alive
36 „	—	50 % „	—	—	—
48 „	10 % alive	50 % „	2-3 % alive	Few alive	—
60 „	—	25 % „	—	—	All dead
72 „	Very few alive	10 % „	—	—	—

Conclusion.—The large percentage of deaths amongst larvae exposed in cultures of faeces is explained by the rapid decomposition which takes place at so high a temperature, even when charcoal is added in large amount. In some instances the larvae were dead within seven hours, whilst in others they survived for twelve and eighteen hours. The comparatively long resistance of some larvae on dry grass can be explained by the formation of clusters, as mentioned above.

42° C.—Cultures with eggs in faeces plus charcoal were attempted on two occasions, but with negative results, whereas control cultures kept at 28° C. showed the presence of mature larvae in three days. In agar medium five attempts were negative, the eggs being found dead in the morula stage within four to six days, whilst the medium itself was in very good condition. In a sixth attempt in agar, in which the eggs were at tadpole and embryo stage, several larvae of the second stage were found moving about on the second day, but they were all dead by the following day. Cultures in agar kept at 42° C. for two hours daily and then removed to a temperature of 28° C. proved positive in several instances.

Mature Larvae.—Mature larvae kept in pure faeces at 42° C. are killed very quickly, owing to decomposition of the medium. The addition of charcoal at this temperature gives better results than at 45° C.

Summarizing the several attempts at preservation in charcoal-faeces at 42° C., I found that—

after 17 hours 85 per cent. of larvae were still living ;

„ 24 „ 80 „ „ „

„ 40 „ 50 „ „ „

„ 48 „ 10 „ „ „

„ 65 „ only a few larvae still survived.

The number had still further decreased three to five days later.

In agar medium the larvae resist much longer. The following are the results of several attempts:—

Examinations at varying intervals showed a very small percentage of deaths during the first two days. On the 3rd day 70 per cent. of the larvae were still alive, on the 4th day 65 per cent. By the 6th day it was

no longer possible to accurately estimate the percentage, owing to disintegration of the larvae previously dead, but, roughly speaking, it seemed as if about 40–50 per cent. survived. Ten days later only about 20 per cent. seemed to have survived. There were still a fair number living on the 14th day, but the survivors had considerably decreased by the 18th day. On the 20th day it was only possible to detect living larvae after a careful search, and on the 26th day it was not possible to find any living larvae on the three different culture plates used.

Biological Remarks.—At 42° C. in agar medium the mature larvae examined under the microscope show a more pronounced motility than those kept at 45° and 37° C. Their movements are particularly noticeable during the first two days, but later about half of the larvae appear lethargic and are very slow to move when awakened. It therefore seems that a temperature of 40°–42° C. gives the maximum stimulation to larvae—for limited periods, at least.

40° C.—Cultures with eggs in pure faeces at 40° C. invariably failed. In cultures made with faeces plus charcoal, I obtained three positive results out of five attempts. The cultures with agar succeeded in the majority of cases, but mortality amongst the larvae amounted to about 50–60 per cent., chiefly in the first stage. The remaining larvae reached maturity within three to four days. Mature larvae resisted for a considerable length of time at constant temperature of 40° C., when kept in a dark moist place.

In these cultures, left at 40° C., some of the larvae which had reached maturity remained alive for 24–28 days.

37° C.—Several cultures were also kept at 37° C. in faeces alone, but out of six attempts two failed, owing to putrefaction, whilst in the positive cases the number of larvae was small. In faeces to which charcoal was added the result was better, but the failures were still frequent and the mature larvae always scarce. In agar cultures the result was nearly always positive. In examining these cultures carefully, I found that larvae of the first stage died to the extent of 40 per cent. Considerable numbers in the second stage also died, but the remainder reached maturity either just previously to, or at, normal time. The mature larvae were more motile than at normal temperatures, and the space between the body and the outer skin was quite conspicuous within four to six days of maturity. In continuing the same agar cultures at 37° C., I observed that after four to six days the mature larvae were still alive, but by the 10th day 60 per cent. had died. Only a few were alive on the 20th day, on which date some of them had lost their outer skin. There were practically none alive after twenty-eight days. It can accordingly be concluded that the maximum time of resistance for larvae at 37° C. is about a month.

Observations in the Field—High Temperatures.

(1) On the 12/3/15 a lot of faeces heavily infected with eggs were placed at the bottom of some stems of grass in the open. During the period of observation they were frequently watered and kept covered with a glass bell 30 cm. high to ensure constant moisture. In the afternoon of the first day, the temperature under the glass bell rose to 56° C.,

whilst in the centre of the faeces a temperature of 45° C. was recorded. During the night the temperature decreased to a minimum of 12° C., and during the two following days there was practically no variation outside the limits described. On the 15/3/15 when the faeces were examined, the eggs were found to be black and dead, whilst a few dead larvae of the first stage were also found.

(2) The second experiment was carried out on the 12/3/15 under similar conditions except that a large amount of charcoal was added to the faeces. The temperature conditions remained the same, and three days later, when the culture was examined, the results were identical with those of the previous trial.

In the case of the first experiment the death of the larvae could be explained by the decomposition of the faeces under the conditions of high temperature, but in the second experiment decomposition was not so far advanced. This result would support that obtained in the laboratory, showing that a temperature of 45° C., acting for more than two hours daily, killed both eggs and larvae.

Conclusions.—Temperatures above 36° C. act more or less deleteriously on the cultures of *Haemonchus contortus*. Mature larvae resist temperatures up to 70° C., providing they are only exposed for a short time. Constant temperatures between 42°–50° C. are resisted by mature larvae from about two hours for the higher degree, to a maximum of a month for the lower degree, but cultures of larvae are killed. The temperature of 40° C. seems to be the highest at which the larvae can grow, but even at 37° C. the mortality amongst eggs and mature larvae is very heavy. As Looss and some other authors have previously noted, decomposition of the medium plays a very important role in the negative results obtained with cultures under high temperatures. The addition of charcoal to the faeces acts as a palliative to decomposition, but cultures in agar seem to be more suitable for the growth of larvae exposed to high temperatures. Temperatures of 37°–40° C. first act on young larvae by overstimulation, with the result that a certain number die, and the remainder reach maturity in the minimum of time. Between 40°–50° C. the mortality is still higher and the surviving larvae are weaker, whilst the time required for maturing is prolonged.

Mature larvae react under high temperatures showing increased motility, followed by a period of inactivity due to exhaustion, in which marked destruction of the intestinal granulations is noted. This period ends in the death of the larvae. The laboratory experiments at constant high temperatures, however, are only of minor importance in regard to the behaviour of larvae under natural conditions, since in the field the temperature oscillates between a maximum in the day time and a minimum at night. Furthermore, in the open, other important factors such as light and dryness, exercise considerable influence and vitiate comparison between laboratory and field experiments. Under the natural conditions of the field there is also the fact that some larvae protect themselves against the sun by migrating to places where they are protected from the direct rays. This point will also be dealt with when considering the migration of larvae.

“Normal” Temperatures: 35° C.—This temperature was generally adopted when dealing with the evolution of the free larvae, and it was found to be very suitable for their growth. The details of some experiments are:—

(1) On the 14/12/13, at 8 a.m., eggs of *Haemonchus contortus* were transferred to a liquid medium and kept at 35° C. At 10 p.m. (fourteen hours later) 40 per cent. of the eggs had hatched. The following evening at 9 p.m. (23–25 hours after hatching) 30 per cent. of the larvae were in the first ecdysis. On the 17th, at 9 a.m., the majority of the larvae were in the second stage, whilst others were in the second lethargus. By 5 p.m. several larvae had reached the third stage. With cultures in faeces or in gelatine the results were much the same, except that wherever marked decomposition of faeces occurred a number of larvae died in the first or second stage. At this temperature the mortality is very small and the growth of the larvae in liquid medium is so uniform that very few immature specimens are found on the fourth day. After reaching maturity, the majority of the larvae immediately commence “swimming” movements in a very pronounced way, and show the optimum of vitality. The migration up the walls of culture jars can be easily seen by the naked eye on the 4th day.

28°–32° C.—In cultures grown at this temperature the larvae reached maturity within three days, frequently to the extent of 90 per cent. In jar cultures 30 per cent. of them were seen to be crawling over the walls, reaching 3–4 cm. above the medium. On the 4th day the wandering of the larvae on the walls was very conspicuous. On examining larvae taken from these cultures under the microscope, the typical swimming movements were clearly seen, showing that the larvae were in the optimum of vitality.

25° C.—Faeces containing eggs from the morula to the embryo stage showed larvae of the first stage, first lethargus, and a few of the second stage, within fifteen hours. On the 3rd day 20 per cent. of the larvae had reached maturity, but they had not yet commenced crawling up the walls of the glass. By the 4th day, however, a heavy migration was usually noticed.

20°–22° C.—Temperatures between these two limits were recorded in the laboratory during the greater part of April, 1915, during which time several cultures in agar or in faeces were left in the room, but exposed to only a weak diffused light. By the 2nd day, a few larvae in the agar cultures were in the advanced second stage, but were rather poor. In cultures in faeces 80 per cent. of the larvae were in the advanced second stage, and were rich in granulations. After three days a very few mature larvae were occasionally seen in agar, whilst in the faeces 40 per cent. of the larvae were usually mature. On the 4th day many larvae had migrated from the faeces on to the walls of the jar, but they were not so numerous as in the case of cultures kept at 25° C.

From these notes it can be concluded that the larvae of *Haemonchus contortus* can grow without showing any marked variations between temperatures of 22°–35° C. These two figures, however, represent fairly well-defined limits, since at 20° C. the migration of the mature larvae on

to the walls of the culture jar is less rapid and at 37° C. they frequently seem to suffer from over-stimulation and exhaustion by loss of their granulations.

Low Temperatures, 15°–18° C.—Temperatures between these figures were frequently recorded in the laboratory during May, 1915. Infected faeces slightly spread out in water and kept in diffused light showed the following progress in development after a lapse of three days—expressed as percentage of uninjured eggs originally present.

Eggs in the tadpole stage 10.

Larvae in the first stage 10.

Larvae just past the first lethargus 40.

Larvae in advanced second stage 40.

After four days no larvae were detected on the walls of the culture glasses, but two days later a few larvae had reached maturity, and were found ascending.

15° C.—Infected faeces kept in glass boxes showed the following development after four days:—

20 per cent. eggs in the tadpole stage;

50 per cent. larvae in the first stage;

30 per cent. larvae in the second stage and rich in granulations.

After eight days numbers of mature larvae were seen crawling on the walls of the glass.

At a temperature of 15° C., occasionally descending to 13° C., a culture of faeces kept in a glass box showed on the 12th day a few larvae crawling up the walls.

8° C.—Infected faeces placed in a rectangular glass vessel were stored in a dark cold store-room. *On the 10th day* all the eggs were in the morula and in the tadpole stage. A part of the faeces was put up for culture, and after three days 30 cent. of the eggs were found to be dead, while 60 per cent. developed. *On the 18th day* and again on the *21st day*, examination showed that 70 per cent. of the original eggs were dead, while 30 per cent. had developed to larvae in the second stage. These latter were apparently dormant, but gradually awakened under the microscope. From this time onwards the temperature in the cold storage changed, and the observation was discontinued.

4° C.—Infected faeces showing eggs in the morula and tadpole stage were collected, placed in glass boxes, and stored in a cool room, at a constant temperature of 4° C. On examining a specimen lot after forty days, eggs were found at the same stage as when stored. Cultivation of these was then attempted, but after ten days of observation no hatching embryos or developing eggs were detected. On the 50th day the remaining faeces were placed in an incubator, but no progress in the development of the eggs could be detected on subsequent observation.

0° C.—To test the resistance of eggs at this temperature, some observations were made by putting a layer of infected droppings between two

blocks of ice. After varying intervals the faeces were put up for culture and four days later the following results were noted:—

20 hours' exposure. A few eggs were found dead, but the majority developed.

22 hours' exposure. Culture showed 90 per cent. mature larvae and 10 per cent. dead eggs.

24 hours' exposure. Culture showed 50 per cent. of mature larvae and 50 per cent. of dead eggs.

36 hours' exposure. Only two incompletely developed larvae hatched out.

48 hours' exposure. Only dead eggs were found.

Mature Larvae. Pieces of blotting paper were soaked in water containing mature larvae and afterwards placed in a Petri dish. The Petri dish was stored in the refrigerator between two blocks of ice.

After 1 month's exposure. 80 per cent. of larvae showed movements as soon as they were placed under the microscope. The remainder were dead.

After 3½ months' exposure. On immediate examination the larvae were motionless. Examination of the same lot 12 hours later showed 60 per cent. to be living.

After 4½ months' exposure. An examination made 12 hours after the larvae were picked out showed 30 per cent. to be living. 70 per cent. were dead, all of which had large vacuoles in the cells of the chyle intestine.

After 6 months' exposure. An examination made 24 hours after removal showed 5–6 per cent. of living larvae with the cells of the chyle intestine poor in granulations and with large vacuoles.

After 7 months' exposure. The few remaining larvae in the Petri dish were dead.

–2 to –3° C.—Observations were made by exposing infected faeces in the field during the winter time. During the course of five days' observation, a temperature of –2° C. was registered for three nights and a temperature of –3° C. for the remaining two nights. These temperatures remained fairly constant for six hours. Cultures were made from the faeces after exposure, and kept at 25° C. A fair number of mature larvae were detected three days later.

–9.5° C.—This temperature was also recorded in the field during the night time, and presumably lasted for some hours. Cultures made subsequently at 25° C. showed the presence of mature larvae within four days.

Temperatures Alternating from Below Normal to Normal.—In the field the larvae are rarely subjected to constant temperature for any length of time. During the South African summer the daily maximum

is unduly high, while the night temperature closely approximates that best suited to larval development. During the winter the daily temperature approximates the optimum, but the evening temperature is unfavourably low. A few observations upon the influence of alternating temperatures are therefore of interest.

Larvae at the First and Second Stage Exposed to a Sudden Decrease of Temperature.—A quantity of faeces from one infected sheep was divided into three batches, and each batch cultivated in pure faeces and in agar. These six cultures were placed at 24°–25° C. The following day larvae in the first and second stage were found to be present. One batch was then placed at 0° C., the second one at 4° C., and the third at 10° C. Twenty-four hours later the cultures were removed and again kept at 24°–25° C. The results obtained on the two succeeding days of observation are summarized in the following table:—

Day of Observation.	Cultures at 0° C.	Cultures at 4° C.	Cultures at 10° C.
1st day after exposure	<p><i>Agar</i>—Few larvae in second stage</p> <p><i>Faeces</i>—20 per cent. larvae in second stage</p> <p>In both cultures numerous larvae dead in first stage</p>	<p><i>Agar</i>—70 per cent. larvae in second stage, not well developed</p> <p><i>Faeces</i>—70 per cent. larvae in second stage, well developed</p> <p>Larvae dead in first stage in both cultures.</p>	<p><i>Agar</i>—70 per cent. larvae in second stage, well developed.</p> <p><i>Faeces</i>—80 per cent. larvae in second stage, 5 per cent. larvae mature.</p>
2nd day after exposure	<p><i>Agar</i>—No mature larvae</p> <p><i>Faeces</i>—Few mature larvae</p>	<p><i>Agar</i>—No mature larvae</p> <p><i>Faeces</i>—Few mature larvae</p>	<p><i>Agar</i>—Some mature larvae.</p> <p><i>Faeces</i>—Numerous mature larvae.</p>

Conclusions.—No cultures died from exposure. In exceptional cases the larvae reached maturity in the usual time of three days, but on the 4th day larvae were found as usual on the walls of the glass in all three lots. In general, therefore, both eggs and larvae of *Haemonchus contortus* seem to resist sudden decrease of temperature, although larvae in the first stage seem to be peculiarly susceptible and numbers of them die.

Daily Decrease of Temperature during Development of Eggs and Larvae.—A similar experiment with three batches was carried out, but instead of a single continuous exposure of 24 hours, an intermittent exposure of 6 hours

was given on three successive days, with storage at 25° C. in the 18-hour intervals. Results :—

Day after Collection.	Cultures at 0° C.	Cultures at 4° C.	Cultures at 10° C.
1st day	<i>Faeces</i> —Few larvae in first stage	<i>Faeces</i> —80 per cent. larvae in first stage, very few in second stage	<i>Faeces</i> —80 per cent. larvae in first stage, 10 per cent. in second stage.
2nd day	<i>Agar</i> —Few larvae in second stage <i>Faeces</i> —70 per cent. larvae in second stage	<i>Agar</i> —70 per cent. larvae in second stage <i>Faeces</i> —90 per cent. larvae in second stage	<i>Agar</i> —80 per cent. larvae in second stage. <i>Faeces</i> —70 per cent. larvae in second stage, 20 per cent. in second lethargus.
3rd day	<i>Faeces</i> —Few larvae in second lethargus	<i>Faeces</i> —40 per cent. mature	<i>Faeces</i> —70 per cent. larvae mature.
4th day	<i>Faeces</i> —Some mature larvae	<i>Faeces</i> —70 per cent. mature larvae	<i>Faeces</i> —90 per cent. mature larvae.

Conclusions.—In cultures kept between 4° C. and 10° C. the departure from normal development is small, but marked differences are noticeable in the cultures kept at 0° C. Young larvae exposed for prolonged periods suffer to a greater extent than those exposed for a short period daily.

Trials were also carried out with agar cultures alternating daily, for six days, between 28° and 10° and between 28° and 0°. In the first case the proportion of larvae subsequently developing at 25° C. was relatively high, but in the second case only two or three larvae were found alive. In consideration of the fact that development at low temperatures is more favourable in faeces than in agar, it is safe to assume that the results would have been better in faecal medium and that even at the lower range the larvae would grow although the percentage reaching maturity might be small. In these experiments a transition period at 15° C. was allowed in transferring cultures from 0°–4° C. to 25° C. In the field the slower and more gradual transition from maximum to the minimum of temperature would favour the easier development of the larvae.

Field Experiments.

Cultures at alternating temperatures were also attempted in the field under different climatical conditions, eliminating as far as possible the action of dryness and light.

(1) Maximum 56° C.—minimum 12° C.

This case has already been detailed under the heading "High Temperatures—Observations in the Field."

(2) Maximum 26° C.—minimum 15° C. On the 15/3/15 faeces from the same sheep as used in experiment (1) were placed amongst grass under the shade of a tree. The culture was covered during the day time, but left uncovered during the night time, and was constantly kept moist. In the evening of the 4th day mature larvae were found crawling on the grass. The temperature by day provided optimal conditions, but the low temperature at night retarded slightly the migration of the larvae on to the grass.

(3) Maximum 26° C.—minimum 12° C. On the 26/3/15 a culture was placed under conditions similar to (2). The maximum temperature recorded during the next six days was 26° C., whilst at night time the following figures were recorded as minima:—12·5°, 15°, 14°, 13°, 12·5°, 12°, and 12·3° C. On the 4th day the most advanced larvae were still in the second stage, whilst on the 6th day 10 per cent. had reached maturity, and only on the 7th day were the larvae found crawling on to the grass.

(4) Maximum 26° C.—minimum 4° C. On the 15/4/15 a culture was placed under the same conditions as before. The temperature on the first night of exposure fell to a minimum of 8·5° C., and only slight variations were recorded in the following ten nights. The first larvae to hatch were only detected on the evening of the 2nd day; by the following evening 90 per cent. were found in the beginning of the second stage. On the 10th day 10 per cent. of the larvae were mature, and some were crawling on to the grass. 30 per cent. of the larvae were found dead in the first and second stage. From the 10th to the 15th day the culture was allowed to lie uncovered and not watered, the minimum temperature recorded during the night time being 4° C. Per hundred examined, 50 were now found as dead larvae of the first and second stage, 40 were alive in the first and second stage, and the remaining 10 had reached maturity.

It appears, however, that with a minimum in temperature of 8° C. the development to maturity is retarded until the 10th day, whilst the mortality amongst larvae is fairly high. The minimum of 4° C., combined with the progressive dryness, practically stops development of the larvae, and about one-half of the larvae die. The first portion of this experiment differs noticeably from the results obtained from the laboratory experiments referred to above, where the cultures were exposed daily to a temperature of 4° C. and 10° C.

(5) Maximum temperature 26° C., and minimum temperature -8° to -10° C. On the 11/6/15 infected faeces were placed in the open, amongst dry grass 10 cm. high and not very thick, generally resembling that found in the veld grazed on by cattle during the winter time. The culture was constantly kept moist, covered during the day time by a large glass bell and protected from the direct sunlight. Between sunset and sunrise the culture was uncovered. From the 11th to 13th June, the minimum temperature recorded during the night was -7° C., whilst the maximum under the glass bell during the day time was 26° C. On the 13th June, the culture showed eggs in the morula and embryo stage. In a sample placed

in culture at 35° C., the larvae reached maturity within three days. From the 13th to 17th June the minimum temperature was -5° C. to -7° C. On the 17th June a sample culture showed 50 per cent. of eggs unhatched, the remainder being larvae of the first and second stages. On the same day faeces of the same batch, placed near the above culture, on the 11th June (but left unprotected during the day time and not watered) were found not completely dry, but did not show larvae. A sample of the latter faeces cultivated at 35° C gave negative results during the three days of observation. From the 17th to the 23rd June the minimum night record varied from -5° C. to -8° C., the latter only being recorded on one night. On the 23rd June 90 per cent. of eggs had hatched, the larvae having reached the end of the second stage and being rich in granulations. Up to the 28th June the minimum temperature varied from -6° C. to -8.5° C., but on this latter date no larvae were found on the grass. On the more exposed pellets a few eggs containing embryos were found dead. 40 per cent. of the lot were dead larvae in the second stage, and 60 per cent. were living larvae in the second stage, but were not rich in granulations. No mature larvae were found. Up to the 5th of July the minimum temperature during the night was about -6° C., except for one night, in which -10° C. was recorded. On the 5th July very few larvae were found in the faeces, and it may be that the larvae found shelter in the ground. On the 8th July, after a rain of twenty-four hours and with a temperature of 10° C., a number of larvae in the second stage were found in the faeces, and six mature larvae were found on two grass blades. On the 15th July, after a night of rain and with a temperature of 14° C., eighty mature larvae were found on three grass blades.

Temperatures in the Field.—In considering first of all the high temperatures, the maximum recorded in South Africa under the conditions of cloudy, moist weather cannot be considered to either prevent the development or to kill the larvae of *Haemonchus contortus*. In sunny weather when the ground is moist, the first observation to be recorded is that larvae lying in wet earth or in faeces are not necessarily exposed to the same maximum of temperature of the atmosphere. On the 17/3/15 I examined a culture in the field that had been kept covered with a glass bell at a temperature of 52° C., whilst at a depth of 3 cm. between the faeces and the earth the temperature was 44° C. On the 26/6/15 the temperature was 43° C. in the sun, whilst in wet cotton wool kept in a glass box the temperature was 22° C.

The second observation, referring to effect of moisture of the soil in sunny weather, is that in moist surroundings the larvae are able to escape from the direct sunlight and reach shelter in cooler places. I will refer to this point again shortly. It must be added that notwithstanding the above instinct of seeking for shelter, numbers of larvae are killed in the first stage, so that it appears that the migratory instinct at that stage has still only a slight effect. It seems to be justified to deduce that in hot weather and on moist veld a high temperature does not have much effect on the larvae of *Haemonchus contortus* when in the second stage. With regard to the eggs, the hot moist temperature of the veld hastens the hatching of the embryo, but if the temperature of the ground rises above

42° C. and remains at that point for several hours, the eggs are killed. If the air and earth are dry, the eggs are killed by temperatures higher than 42° C. This conclusion is made under the supposition that only the temperature was responsible, but in practice it will be found that complete reliance cannot be placed on this factor. Under natural conditions mature larvae may be exposed in dry weather to direct contact with the atmosphere, or they may be sheltered in faeces or in earth. In the first case individual larvae that have crawled on to grass stems will naturally be subjected partially to the action of the temperature and to the more direct action of the sunlight. The influence of temperature on larvae is subordinated to the action of sunlight to such an extent that no deduction could be drawn from the experiments just referred to.

For reasons which will be discussed later, I may state here, that when in dry weather the temperature reaches about 42°–45° C., the larvae exposed on the dry grasses are killed within ten days. In the second case where the larvae are sheltered, their resistance increases against heat of the sun. One observation on this point was reported when discussing the resistance of larvae to a temperature of 50° C., but more observations are wanted. In hot, dry weather, however, the temperature causes such marked desiccation of the faeces and of the superficial layers of the ground that deductions as to the action of the temperature alone are not possible. It can only be said that the high temperatures experienced in the veld during the summer time undoubtedly help to kill a large number of larvae, but at the same time many of these larvae can be killed before the maximum temperature has been reached. Finally, it may be stated that the average summer temperature of South Africa is very favourable for the development of the larvae of *Haemonchus contortus*.

Concerning the low temperatures experienced in South Africa, it may be said that the normal spring and winter temperatures do not prevent the development of the eggs or larvae, but prolong the period required before the larvae reach maturity. The resistance to adverse conditions of eggs and larvae and their ability to develop, considered in relation to the effects of temperature, in the winter season of South Africa, depend chiefly on the absence of uninterrupted spells of low temperature; the minimum, even if occasionally very low, only lasts for a short time during the night, whilst in the day time the average is the optimum temperature required for the growth of larvae. The above considerations apply to an even greater extent to the preservation of mature larvae, as in working with the resistance of eggs and larvae I came to the conclusion that in a moderate, cold ambient the larvae remain in a better state of preservation and for a longer period than in warm weather.

During the three years in which these investigations were undertaken, I could not detect in any instance larvae that were killed as a result of exposure to cold weather in the veld, if they were allowed to find shelter either in faeces or in the soil. Unprotected larvae would not survive the winter season for reasons other than cold, and though they might be sheltered in pellets lying on the surface of the ground, yet this would not be sufficient protection. The possibility of the mature larvae reaching the host is practically excluded in winter time, owing to the dry weather, during which the larvae cannot travel. Observation No. 5 in the field

under the heading of "Low Temperatures," shows that on a rainy day in winter time the infection of sheep is feasible.

Moisture.

The conditions of moisture under which eggs and larvae are found in faeces will be dealt with under the headings of "Moist," "Wet," and "Soaked Faeces."

Moist Faeces.—Faeces contain a sufficient amount of moisture to preserve the same consistency as when passed by a healthy sheep. This is the case when fresh droppings are placed in a glass vessel, or when dry droppings are artificially moistened with water, or in the field naturally by rain.

Under any of the above conditions the growth of the larvae is normal. They represent the optimum condition of moisture for the development of larvae.

Wet Faeces.—The moisture reduces the faeces to a pulp. As in the case of diarrhoeic faeces, mentioned previously, the eggs do not hatch or the larvae die shortly after hatching. The failure is due either to the presence of toxins or the density of the medium produced by the moisture. Attempts at cultures in pellets infected with numerous eggs and mixed with a fair amount of water to reduce them to the consistency of pulp always failed. This manipulation reduces or destroys the porosity of the faeces and thus prevents the circulation of air; consequently the insufficient supply of oxygen and the fermentation of the medium are noxious for the growth of the larvae. In the field these conditions are practically never met with, because even in the case of prolonged rain the necessary porosity and thus the air supply is always maintained, allowing the growth of larvae.

Soaked Faeces.—The eggs or larvae are completely immersed in water. In this case also the layer of water necessary to cover the pellets is too thick to allow of either the required air supply or of the escape of the products of fermentation. Consequently the eggs and larvae die as is the case in the pultaceous media. The same may be said of the eggs that are washed out from faeces and transferred to a liquid medium, in which case the thickness of the layer of water must be taken into consideration. The following experiments were carried out in connection therewith.

(1) In a series of six glass tubes, with a diameter of 2 cm., eggs in the morula and tadpole stage obtained from different sheep were placed at the bottom and covered with 2 cm. of liquid medium. The tubes were left in a slightly diffused light, at a temperature of 24° C. to 30° C.

On the 7th day no progress was noted in the eggs, when a number of eggs were transferred to agar culture. After three days some larvae were found in the first stage in the agar cultures, and after six days very few larvae had reached maturity. The majority of eggs and larvae at the first stage were dead. The remainder of the eggs in the glass tube did not show any development in further examinations after several days and were found dead.

(2) Another series of cultures in six glass tubes, with a diameter of 2.5 cm. and a depth of water of 2 cm. were kept under conditions similar to those in the previous experiment, and gave the following results :—On the 4th day larvae in the first stage were found ; on the 7th day numerous larvae in the first stage were found ; on the 10th day 80 per cent. of larvae in the first stage were found dead.

On the supposition that the smallness of the diameter of the tube prevented a sufficient change of air in the culture, I made the following experiments :—

Eggs were placed under the same conditions as before, but the liquid medium was 8 cm. high, with a tube diameter of 9 cm.

On the 4th day nearly all the eggs had hatched, and the larvae had reached the first stage.

On the 7th day the larvae were still in the first stage, and 50 per cent. had died. The remainder were extremely poor in granulations. Some of these larvae were placed in agar cultures, and three days later 50 per cent. had reached maturity ; the remainder had died.

On the 10th day the larvae still present in the liquid medium had died.

In further experiments the thickness of the water was reduced to 2 cm. and the diameter increased to 11 cm., but fifteen days later all the larvae were found dead in the first stage, and extremely poor in granulations.

Another series of observations was made with liquid cultures 1.5 cm., 1 cm., and 0.5 cm. deep, with a tube diameter of 11 cm., and kept at a temperature of 25° C. On the 3rd day 20–30 per cent. of eggs were found unhatched, and the remaining 70–80 per cent. were free larvae in the first stage, the majority being motionless. On the 10th day very few larvae had reached maturity in the culture 0.5 cm. deep, and were poor ; the remainder had died in the first stage. When the liquid medium was only 1–3 mm. high, the larvae hatched and grew in the same time as in faeces.

I used these liquid cultures for three years, chiefly in dealing with the stages and structural changes of larvae in the outer world, and the method and results are more extensively dealt with under “ Cultures of the Larvae.”

If the temperature is lower than “ normal,” the liquid cultures do not show such rapid progress, and the larvae are poorer than in cultures in faeces made at the same temperature.

Conclusion.—In liquid medium kept immobile and deeper than $\frac{1}{2}$ cm., a rather small percentage of the eggs hatch after four days, but the larvae make very little progress, and within twelve to fifteen days death occurs, presumably owing to lack of air. The growth of the larvae is possible and normal when the liquid medium is in a thin layer of a few millimetres.

Mature Larvae in Liquid Medium.—In this connection the following experiments were recorded :—

(1) Tap water, not changed, kept in a diffused light at a temperature of 28°–32° C.

On the 21/10/13 young mature larvae were immersed in water 3 cm. deep in an evaporating dish. All larvae were moving quickly. On the 22/10/13, 50 per cent. of the larvae were coiled up at the bottom of the dish, the remaining 50 per cent. were still swimming. On the 25/10/13,

90 per cent. of the larvae were coiled up; 10 per cent. were stretched out and dead. On the 25/11/13 the conditions were unchanged, but two minutes after being placed under the binocular microscope, the larvae which were coiled up began swimming.

(2) Larvae kept under similar conditions to those in the previous experiment, but in river water. The average temperature was about 32° C. during the first three months, and 25°–28° C. during the remainder.

On the 9/11/13 the larvae were immersed in water. On the 20/12/13, 5–10 per cent. were stretched out dead, with big vacuoles in the chyle intestinal cells. The remainder were coiled up, and awakened after five seconds' exposure to light under the binocular microscope. On the 9/1/14 there was no change. On the 3/3/14, 80 per cent. were still coiled up, showing slight movements after two to three seconds' examination under the binocular microscope. On the 4/4/14, 50 per cent. were still coiled up, but they awakened when exposed to light. The remainder had undergone the second ecdysis, and were stretched out and dead, but did not show any remarkable internal structural changes. Numerous outer envelopes were present in the water. On the 20/4/14, 5 per cent. were still coiled up; the remaining 95 per cent. had passed the second ecdysis and were dead.

(3) The larvae were placed in a graduated tube, with a diameter of 6 cm., containing tap water 20 cm. deep. The average temperature was 28° C. during the first month, decreasing later to 19°–20° C.

On the 7/4/15 the larvae were placed in water. On the 8/4/15 the majority of larvae were moving at the bottom of the tube. On the 11/4/15 to the naked eye the mass of larvae appeared motionless. On the 11/5/15 about 10 per cent. were dead, with the outer envelope present, and large vacuoles in the chyle intestine; the remainder showed swimming movements. Some of them had passed the second ecdysis, were swimming, and the internal structure had not changed; they were rich in granulations. On the 18/6/15, 90 per cent. of larvae were without the outer skin, coiled up, and awakened under the light of the microscope. Only 2–3 per cent. of the larvae without capsules were dead; a few larvae with the outer skins were also dead. On the 15/7/15, 90 per cent. of larvae were without the outer skins; only 5 per cent. of these were dead. Some larvae were allowed to dry on a glass slide, and after twenty-four hours a little water was added. Six hours later all the larvae without the outer skins were dead, and only a few with the outer skins were alive. On the 20/8/15, 70 per cent. of larvae were alive, and of them 90 per cent. lacked the outer skin. Of the dead larvae more than half were without skins. On the 9/9/15, 50 per cent. of larvae were alive; 95 per cent. of the living larvae were without the outer skin.

From the above observations, it appears that in water mature larvae of *Haemonchus contortus* find a suitable medium for preservation. The small percentage of deaths occurring during the first few days is also observed in other media. In the following days the larvae are observed to be coiled up; they keep practically motionless, and are rich in granulations and well preserved. From the first month onwards the larvae undergo the second ecdysis. A large percentage of larvae die during the fourth month of preservation, and a small percentage live up to the fifth month.

Taking into consideration the pools of water in the field, it appears that the mature larvae in these reservoirs find a good ambient for their preservation, and to a certain extent a favourable place for infecting the host. In fact, larvae present in pools 20 cm. deep are preserved when lying at the bottom, or when sheltered under the more superficial particles, where they are not hurt by light. As it will be seen later, in dealing with the action of currents in the water under the effect of temperature, changes take place, by which the larvae are able to reach the surface of the water and can be taken up by the sheep. Infection of sheep also takes place when a flock stirs up the water and brings the larvae to the surface. These points go to show that the infection of sheep by infected pools is feasible.

Behaviour of Mature Larvae in Moist and Liquid Media.—On the 11/7/15 a large number of young mature larvae, in pellets of faeces mixed with charcoal, were heaped up in a glass dish containing water 1 cm. deep and kept in a diffused light. At the same time, by means of a piece of blotting paper, numbers of larvae were collected from the walls of the jar containing the above culture. The blotting paper was placed against the internal walls of a test tube, with the lower portion immersed in the water and the test tube was kept in a cupboard. On the 18/7/15 a large number of living larvae were found in the water, whilst in the pellets only a very few were found.

In the test tube half the larvae were found in the water, the other half coiled up on the blotting paper.

In conclusion, it can be considered that mature larvae prefer pure water to faeces, probably owing to the fact that mature larvae usually leave the faeces when they have reached maturity. From the experiment with the blotting paper, however, it appears that the larvae have no decided preference for water when another moist suitable medium is available. Some similar observations are recorded on page 395.

Desiccation of the Larvae.—The removal of water from the larvae can be performed in an effective manner by submitting the larvae to the action of a desiccator. Personally I used calcium chloride as an absorbent, as this chemical compound removes the moisture in a gradual way without hurting the vitality of the larvae too much. It is admitted in chemistry that after the action of the calcium chloride desiccator 2–3 per cent. of moisture is still present. It stands to reason that a higher percentage of water remains in the tissues of the larvae, which are well protected by a double chitinous envelope.

A second method for the mechanical removal of water from the larvae is to expose them to the free air. This is the method which is most similar to the conditions under which free larvae exist. In order to distinguish between larvae kept in the desiccator and those exposed to free air, the latter will be termed “Dry larvae,” while the former will be referred to as “Desiccated larvae.”

Some observations were made in the laboratory and some on the veranda, where the conditions were practically the same as in natural conditions under trees or in any other shaded spot. Some observations were also made under trees in the open.

The larvae were exposed on a glass slide in faeces or on grass.

In the following paragraphs are included the experiments in which dryness played a predominant rôle. It was ascertained in the course of these investigations, that the resistance of larvae to the withdrawal of moisture varies according to whether they are exposed singly or in clusters or whether they are sheltered in or under some solid substance, and these facts have been taken into consideration in the general arrangement of the experiments now detailed.

The Appearance of Dead and Living Larvae under the Process of Desiccation.—I was unable to define clearly the difference between the two above kinds of larvae, notwithstanding that the following peculiarity appeared to be constant: In the living larva the chyle intestine is well-defined and the granulations of the cells have a glistening silvery appearance. In the dead larva the edges of the chyle intestine are rather indistinct, the granulations are very fine and slightly yellowish. If the larvae are again immersed in water, the living ones clearly show the internal structure with a few small vacuoles in the chyle intestinal cells. In the dead larvae the internal structure has nearly disappeared and the chyle intestinal cells contain numerous large vacuoles. It appears that larvae are killed by desiccation as soon as the granulations stored in the cells of the chyle intestine are exhausted.

Mechanism of Desiccation in the Larval Body.—In following the process of desiccation under high magnification, I observed that each layer, anatomically distinct in the larva (outer skin, inner skin, muscular coat, etc.), becomes contracted and collapses on to the inner one, giving the larva a wrinkled appearance. Consequently, during the process of drying the resistance of the tissues increases as each contracting layer becomes more impervious to moisture. The desiccation of the chyle intestinal cells, which seems the most fatal process, is thus prevented by the external contracted layers of tissues, furthermore a certain amount of fat is present in the protoplasm of the intestinal cells which evidently retards the desiccation of the same. This fatty substance is easily seen under the microscope in breaking a fresh larva on a glass slide in a drop of water, when numerous small particles of fat can be seen scattered about. In examining transverse sections of fresh larvae, the fat drops are frequently seen, sometimes being so numerous or so large that they interfere with the examination of the section.

Eggs and Immature Larvae exposed in Faeces of the Host.

(1) On the morning of the 22/6/15, infected faeces collected in a bag during the night were placed in a current of air to dry, and during the following night were kept in a desiccator. Some faeces collected from the rectum of the same sheep were at the same time placed in a room and some on the veranda.

From cultures made from the three lots of faeces in the days hereafter noted, the following results were obtained three days after inoculation:

On the 25/6/15 in faeces from the desiccator 2-3 larvae for every 100 dead eggs were found. On the 26/6/15, in faeces from the desiccator 2-3 larvae for every 100 dead eggs were found. On the 29/6/15, in faeces

from the desiccator 2-3 larvae in the whole culture were found. In faeces in the room and on the veranda all eggs were dead. On the 5/7/15 and 10/7/15, the three lots of faeces gave negative results on culture.

NOTE.—The sheep from which the faeces were collected was sheltered in a stable during the night, and the faeces found in the morning were slightly warm. The temperature during the first day of exposure was from 10°–12° C.

(2) On the morning of the 4/7/15 infected faeces were removed from the rectum of a sheep and placed in a draught to dry. In the evening they were divided into three lots; one lot being placed in the desiccator, another on the veranda, and the third one left in the room. On the 6/7/15, a culture was made from each lot of faeces, with negative results. Negative results were also obtained from cultures made on the 10/7/15. The temperature during the 1st day after collection was below 15° C., and the air was very dry.

(3) On the 15/4/15 infected faeces dropped during the previous evening and still containing a fair amount of moisture were placed in a desiccator.

A culture, made eight days after, gave a fair positive result, numerous eggs and larvae of the first stage being dead, and some mature larvae were alive.

On the 23rd day a culture was made and very few larvae were found. One month later very few larvae were present in the culture.

NOTE.—The temperature of the room in which the faeces were kept from the 14th to the 15th was about 16° C.

(4) Another experiment on the desiccation of eggs was undertaken, which I will provisionally group with the above one.

On the 8/5/15 infected faeces collected from the rectum were placed in a desiccator without having been previously dried in the air. The temperature in the room was 19° C. For twelve hours the internal walls of the desiccator appeared to be foggy, probably owing to the fact that at that temperature the moisture was not absorbed quickly enough by the calcium chloride.

Cultures were made from this sample of faeces on the 2nd, 8th, and 16th days of desiccation and gave positive results.

(5) Eggs in the embryonic stage.

On the 26/3/15 faeces containing eggs with embryos measuring two to three times the length of the egg were placed on the veranda where the rays of the sun could not reach them. On the 6th day the pellets were quite dry and some of these were placed in water, when they were found to contain a fair amount of dead larvae in the first stage and some in the beginning of the second, which had hatched during the drying up of the faeces. A culture of the same faeces contained 50 per cent. mature living larvae. Some more cultures were made in the following days, and on the 10th day 15-20 per cent. of mature larvae were found. By the 15th day there was only 1 per cent. of mature larvae and on the 20th day only 1-2 larvae could be seen in the whole culture. On the 25th and 28th days the culture was negative.

During the twenty days of observation there were only ten fine days; the remainder were either cloudy or rain fell for a half or whole day. The

minimum temperature was 8°–12° C. at night, and the maximum 35°–40° C. in the sun.

Similar experiments to No. 5 were carried out during the same season by exposing infected faeces on the veranda, the result being that on the 20th day practically all the eggs were killed.

Conclusions.—In the first experiment the eggs in the faeces passed during the night had time to undergo development and some reached the embryonic stage before being exposed to the current of air. The same observation can be made on the eggs used for experiment No. 3. In the fourth experiment the eggs placed in the desiccator had also time to undergo development, as in the first twenty-four hours the action of desiccation was apparently weak. In the fifth experiment, the eggs were allowed to undergo embryonal development before being exposed to dryness. From the figures quoted it appears that in the above cases the eggs showed greater resistance to dryness and desiccation than either the eggs used in the second part of No. 1 experiment or in No. 2 experiment, where the infected faeces were exposed to dryness immediately after being removed from the rectum. In the two last cases the eggs presumably did not undergo development. It can be noted that Looss in working with *Sclerostomes* and *Cylicostomes* found that eggs with mature embryos resisted desiccation better than eggs in which the embryos were not completely developed.

Exposure of Mature Larvae dried up on Glass Slide.—The experiment was started on the 7/4/15. Some slides were exposed in the room and some on the veranda.

Table No. 1.

Date of Observation.	ROOM. (Number of Larvae still Alive.)	VERANDA. (Number of Larvae still Alive.)
9/4/15	Homogeneously spread.....	95 per cent. swimming—rare vacuoles.
11/4/15		0.5 per cent. slow—numerous vacuoles.
12/4/15		All dead, with numerous vacuoles.
13/4/15		" " "
14/4/15		" " "
15/4/15	20 per cent. slow—small vacuoles present	—
19/4/15	10 per cent. slow—small vacuoles present	(In clusters) 2 per cent. swimming—few vacuoles.
24/4/15	All dead—numerous vacuoles....	—
28/4/15	—	(In clusters) all dead.

NOTES.—(1) In order to find whether the larvae were alive they were removed from the cultures and kept in water for twenty-four hours. (2) The large percentage of larvae still alive after two days' exposure on the veranda is due to the conditions of the weather. There was rain from the 7th to the 8th, and it was cloudy in the morning of the 8th. (3) From the above table it appears that larvae exposed to the open air were nearly all killed in four days, while in a room they were all killed after sixteen days. In the veranda few larvae were still alive after twelve days, being protected against dryness. Consequently in the above conditions, a

single isolated larva is killed by dryness, when clustered larvae are still alive.

Exposure of Larvae that had Crawled up on to dry Grass.—The experiment was started on the 17/4/15. The larvae were exposed in a room and on the veranda; at the time of exposure they were ten days old.

Table No. 2.

Date of Exposure.	Larvae still Alive in the Room.	Larvae still Alive on the Veranda.
21/4/15	—	0·5 per cent. swimming—few vacuoles
23/4/15	—	0·5 per cent. slow—numerous vacuoles
25/4/15	Superficial (1) 2 per cent. slow—vacuoles. Clusters, 15 per cent. swimming	—
26/4/15	10 per cent. swimming.....	—
30/4/15	2-3 per cent. slow—vacuoles.....	—
5/5/15	All dead.....	—

A piece of grass blade exposed in the room was immersed in water for a few seconds. Afterwards the grass was placed in another lot of water and left for twenty-four hours. It is likely that in the first lot the superficial larvae only became detached, whereas in the second lot the clusters were freed.

Comparative Result from Exposure of Larvae in a Room, on the Veranda, and in the Desiccator.—The experiment was started on the 24/4/15 with larvae on glass slides and on dry grass.

Table No. 3.

Date of Exposure.	Larvae still Alive in the Room.	Larvae still Alive on the Veranda.	Larvae still Alive in the Desiccator.
25/4/15	Glass—2-3 per cent., swimming Grass—20 per cent., swimming	Glass—1 per cent., few vacuoles present Grass—1-2 per cent., few vacuoles present	Grass—30 per cent. —
26/4/15	Glass—6 per cent., vacuoles numerous Grass—10 per cent., slow; vacuoles numerous	Glass—0·5 per cent. of scattered larvae Grass—1 per cent.....	Grass—10 per cent., slow; vacuoles. —
28/4/15	Glass—0 per cent. of scattered larvae Grass—1-2 per cent., slow; vacuoles numerous	Glass—0 per cent. of scattered larvae Grass—0 per cent.....	— Grass—8-10 per cent., slow; vacuoles.
30/4/15	Glass—10 per cent. of larvae in clusters Grass—0·5 per cent., slow; vacuoles	Glass—0 per cent. of larvae in clusters Grass—0 per cent.....	— Grass—0·5 per cent., slow; vacuoles.
3/5/15	Glass—1-2 per cent. of larvae in clusters Grass—1-2 per cent...	Glass—0 per cent. of larvae in clusters —	— Grass—0 per cent.

NOTES.—The larvae used were five days old. The increase in the percentage of larvae on grass exposed in the room from 30th April to 5th May is possibly due to some clusters being present in the sample tested. The possibility is supported by the difference in living larvae exposed on glass slides in the room from the 28th to the 30th April, when the kinds were separated. For the daily temperature, see page 421.

Comparative Results from Exposure of Larvae in a Room, on the Veranda, in the Desiccator, and in Sunlight.—The experiment was started on the 24/6/15 with larvae on glass slides, grass blades, and in faeces.

Table No. 4.

Date of Exposure.	Larvae still Alive in the Room.	Larvae still Alive on the Veranda.	Larvae still Alive in the Desiccator.	Larvae still Alive in the Sun.
25/6/15	<i>Faeces</i> ... 98 %	<i>Faeces</i> ... 90 %	<i>Glass</i> 30 % <i>Grass</i> 90 % <i>Faeces</i> ... 98 %	<i>Faeces</i> ... 3 %
26/6/15	<i>Faeces</i> ... 95 %	<i>Faeces</i> ... 70 %	<i>Glass</i> 8 % <i>Grass</i> 50 % <i>Faeces</i> ... 50 %	<i>Faeces</i> ... 1-2 %
28/6/15	<i>Faeces</i> ... 90 %	<i>Faeces</i> ... 70 %	<i>Glass</i> 0·2 % <i>Grass</i> 10 % <i>Faeces</i> ... 10 %	<i>Faeces</i> ... 1-2 %
30/6/15	<i>Faeces</i> ... 50 %	<i>Faeces</i> ... 30 %	<i>Glass</i> 0·1 % <i>Grass</i> 3-4 % <i>Faeces</i> ... 2-3 %	<i>Faeces</i> ... 1-2 %
8/7/15	<i>Faeces</i> ... 40 %	<i>Faeces</i> , 20-30 %	<i>Glass</i> 1 % <i>Grass</i> 8 % <i>Faeces</i> ... 0·4 %	<i>Faeces</i> ... 0·2 %
16/7/15	<i>Faeces</i> ... 15 %	<i>Faeces</i> ... 5 %	<i>Glass</i> 0 % <i>Grass</i> 0 % <i>Faeces</i> ... 0·1 %	<i>Faeces</i> ... 0·2 %

In comparing the results obtained in experiments Nos. 1 and 2, it appears that larvae resist dryness equally well on glass as on grass, which was not the result in experiment No. 3. This can be explained by the fact that during the second experiment the weather was very dry. (See page 145.)

In comparing the mortality of larvae in the three different experiments, an increase is noted from the first to the third experiment. This mortality seems to correspond to the weather condition prevailing at the time; during the period of the first experiment it rained on the first

two days; during the second experiment the atmosphere was drier than normal, whilst during the third experiment the first two days, on which the mortality was very high, were warm and dry, with a strong wind blowing.

This observation helps to explain the apparent discrepancy between various observations. The resistance is due to the varying conditions of the ambient, to which the larvae are very sensitive.

In the third and fourth experiments the mortality amongst larvae in the desiccator was not as high as on the veranda, and during the first few days was even lower than in the room. This fact is explained by the strong current of dry air acting on the larvae in the open in the Transvaal. In the desiccator the space is limited, ventilation is absent, calcium chloride acts rather slowly, and there is but little light present. The final result obtained in the desiccator consists of a minimum of moisture, which is never reached in the open air; thus the big mortality of larvae in No. 3 and No. 4 experiments.

In comparing the third and fourth experiments, it is remarkable to note the low mortality of larvae exposed on grass in the desiccator as recorded in the fourth experiment. This observation illustrates the role played by the temperature in the phenomenon of desiccation, as the temperature decreased from the daily average of 25°-26° C. in the third experiment, to 10°-12° C. in the fourth experiment.

Observations on dryness in the field.

(1) On the 3/9/14 infected faeces were placed on the earth under a tree so as to be only reached by the rays of the sun for about two or three hours in the morning, and covered with a glass bell. On the 7/9/14 the majority of the larvae had reached maturity, and were mostly found in the earth immediately below the faeces. The glass bell was then removed, and the culture left exposed to the air. On the 24/6/15 several samples of faeces were examined, but no larvae were seen; the earth just beneath the faeces was also examined with negative results.

(2) On the 8/9/14 two cultures were made under the same conditions as above, but were not covered by a glass bell; they were watered several times during the day. On the 12/9/14 the majority of larvae had reached maturity, and as before were found in the earth under the faeces. On the 24/6/15 no larvae were detected in the faeces.

(3) On the 26/3/15 a culture was made under similar conditions to the above. On the 2/4/15 the majority had reached maturity, and the culture was allowed to dry. On the 22/6/15 50 per cent. larvae were found dead in the faeces, with pronounced vacuolation of the chyle intestinal cells. The remaining 50 per cent. were living, with very few or no vacuoles, and with the outer skin intact.

During the above period there was only a slight rainfall on the 7/4/15 and on the 1/5/15 which could not reach the faeces sheltered under the tree. From that date onwards the weather was dry.

(4) On the 15/4/15 another culture was made under similar conditions to the above. On the 30/4/15 10 per cent. of larvae had reached maturity, 40 per cent. having reached the first and second stage.

The results obtained in the above experiments were that a large number of larvae resisted the dry weather for a period of three months when sheltered from the sun, and that no living larvae were found in the same sheltered spot after nine months of exposure.

Do larvae prefer moisture or dryness?—Bohemian beakers containing infected faeces were kept in specimen jars, at the bottom of which were placed thick layers of cotton wool soaked in water. The edges of the beaker were in contact with the inner walls of the jar. The specimen jar was covered with a glass slide. Some of these cultures were left on the veranda, and on examining them two or three months later a few dead larvae were found in the faeces. On the walls of the beaker and on the inner walls of the jar numerous dead larvae were seen. The greatest number of larvae were usually found in the water and on the cotton-wool, some being dead and some still living, with or without the outer skins.

It appears that a layer of water can act as a reservoir for mature larvae.

In gelatine plates used to expose larvae under high temperatures, the larvae were found in the lower layer, where the moisture was more abundant, when the surface of the gelatine was drying. The fact that larvae prefer moisture is shown by their good preservation in water.

In a number of experiments carried out in this laboratory mature larvae lived for five months in water, whilst in dry faeces in the same room eight days later 50 per cent. were dead, and in the desiccator the majority died within four days. The water appears to act as a protection against both sudden changes of the air and desiccation, and, if the layer of water is fairly thick, it protects the larvae from the influence of the sun.

The action of desiccation on mature larvae.—In regard to the action of desiccation on mature larvae, the following conclusions were arrived at:—

(1) The larvae of *Haemonchus contortus* do not resist complete desiccation, and die before this condition is reached, but under natural conditions they resist, (on account of their peculiar structure) the progress of desiccation for a comparatively long time.

(2) The larvae preferably remain in moist surroundings, and by migrating are in a position to seek shelter where the moisture is more constant.

(3) When dryness sets in the larvae gather in clusters, a peculiarity which can easily be observed under the microscope.

On examining a slide, on which larvae were spread in a thin layer of water, the larvae very soon commenced to collect in clusters. With the progress of evaporation of the water only isolated patches were seen surrounding the clusters of larvae. These clusters play an important role in the preservation of larvae in the field against drought; chiefly when the larvae are surprised on grass by the evaporation of dew or rain.

The laboratory experiments on dryness could not be directly applied in the field, owing to the fact that on the one hand the period of dryness in the field is interrupted during the night by the falling of dew, and on the other hand with dryness other important factors, such as temperature

and light, act on the larvae. Nevertheless, dryness plays an important role in connection with the destruction of eggs and larvae in the field.

Dryness during the summer time will be also alluded to in a series of experiments enumerated under the heading of "Light," these experiments being performed in the open and in which the absence of rain played an important part. In this experiment freshly passed infected faeces were placed amongst grass, during a period of drought, but at a time when dew was abundant. If the grass was not high enough the cultures were found to be negative, containing dead eggs and larvae at the first stage, and the pellets of faeces were found to be hard and dry. It appears consequently, that if there is no rain for two or three days very few mature larvae are found in infected faeces. It was also observed that two or three days of cloudy weather were not long enough for larvae to reach maturity, as the evaporation from the faeces takes place so rapidly that the eggs and young larvae are stopped in their development, and later are killed by the first two days of drought.

In winter, eggs and larvae hatch even at a very low temperature in the field if they are constantly kept moist. In this season eggs and larvae are killed chiefly by dryness, as was proved by control cultures carried out at the same time both with faeces kept moist and with faeces drying naturally, the result of which was reported in the paragraph "Temperature." With regard to the manner in which the infection of *Haemonchus contortus* is preserved in the field in the winter time, it was seen that eggs in the faeces or mature larvae on the dry grass or on faeces exposed to the air were killed by dryness in a comparatively short time.

It is therefore necessary to find an ambient in which larvae are able to live through the winter time, or through long periods of drought in summer time, and this ambient is found either in water or in the soil.

Water acting as a reservoir has been mentioned previously. The earth as a reservoir for larvae will be mentioned in the paragraph "Geotropism of the Larvae."

Light.

The effect of light will be dealt with under the headings of *darkness*, *diffused sunlight*, and *direct sunlight*.

Darkness.—Eggs and larvae of *Haemonchus contortus* develop under conditions of light such as are observed during the night time or in an incubator.

Diffused sunlight.—Cultures in faeces do not seem to be the most suitable for the purpose of studying the effect of diffused light on the growth of larvae. In order to demonstrate this point the following experiments were carried out :—

First Experiment.

On the 12/7/15 three cultures on agar were made with eggs washed out from faeces.

- (1) One was placed on the veranda.
- (2) One on the window table in a room.
- (3) One placed in a cupboard.

- (4) Some pellets of the same batch of faeces were placed in a glass dish (in which constant moisture was provided by means of a wet flake of cotton-wool) and kept in the room on the window table.

The average temperature in the room was 18° C., and on the veranda 22° C.

On the 13/7/15 at 5 p.m. the results noted were:—

Veranda: 30 per cent. of eggs had hatched.

Room: The eggs were in the last stages of development.

Cupboard: Some of the eggs had reached the tadpole and some the embryonic stage.

Faeces: On the surface of the pellets were found about 500 larvae in the second stage and in the centre of the pellets about 300 larvae in the second stage.

Another pellet, in which the larvae were probably as numerous as in the above-mentioned one, was placed in a moist glass dish exposed to the sunlight for half an hour, at the end of which time only 10 larvae were found on the surface.

On the 14/7/15 the results noted were:—

Veranda: The larvae were in the first stage and poor in granulations.

Room: The larvae were in the first stage and were poor in granulations.

Cupboard: Larvae in the first and second stages were found, rich in granulations.

On the 16/7/15 the results were as follows:—

Veranda: Poorly developed larvae in the second stage and poor in granulations were found.

Room: Ditto.

Cupboard: 5 per cent. of larvae completed the second stage, and were one-third longer than those on the veranda.

Faeces: All the larvae were in the second stage and well developed.

On the 21/7/15 the results were:—

Veranda: 70 per cent. of the larvae were dead.

Room: Ditto.

Cupboard: 90 per cent. of well developed larvae were at the end of the second stage, 5 per cent. were mature and living, and 5 per cent. were dead.

Faeces: Numerous larvae had reached maturity.

On the 25/7/15 the results were:—

Veranda: All larvae were dead.

Room: Ditto.

Cupboard: 20 per cent. were dead; the remainder had reached maturity. Some were crawling on the walls of the culture.

In the latter part of the experiment the average temperature in the room was 12°–14° C. at night and 16°–18° C. in the day time.

NOTES.—The culture exposed on the veranda was transferred to the room each night.

The number of larvae on the surface of the pellets was determined by soaking the pellet in water and afterwards counting the larvae remaining in the water.

The experiments were repeated several times, with similar results. The following is worthy of note :—

Second Experiment.

On the 4/8/15 a series of agar cultures was exposed, one culture on the veranda, one on the window table, one in a corner of the room where the light could be compared with that of a rainy day, and one on the window table covered with a porcelain dish.

On the 9/8/15 the results were :—

Veranda : All larvae were found dead in the first and second stages.

Window : 30 per cent. of the larvae were found in the first stage and 70 per cent. in the second stage in poor condition.

Corner of room : The larvae were all in the advanced second stage, and were one-third longer than those in the window.

Under porcelain dish : Ditto.

NOTES.—In the first experiment the larvae on the veranda lived longer than those in the second experiment, probably owing to the fact that it was cloudy and raining for four days.

From the above experiment it appears that the larvae of *Haemorchus contortus* do not reach maturity if exposed to a diffused bright light all day. If the diffused light is weak as is noticed in rainy weather, the larvae reach maturity equally as well as they do in darkness.

In faeces exposed to strong diffused light the larvae grow as usual.

It seems that the larvae pass a certain part of their developmental period on the surface of the pellets, when in diffused light.

Direct sunlight.—The action of the sun is a complex one, temperature, dryness, and light all coming into consideration. Some experiments were carried out, excluding as far as possible the action of the first two factors, whilst others were undertaken in the open under natural conditions.

The Action of Sunlight on Eggs and young larvae.—In order to remove every possibility the larvae had of finding shelter from the direct rays of the sun, the following experiment was carried out :—

27/6/15, 11 a.m.—Eggs in the morula stage, obtained by washing out infected faeces, were placed into four Petri dishes, two of them containing a thin layer of agar-agar medium, and two a layer of liquid medium (faeces decoction) a few mm. thick. An evaporating dish was filled with cotton-wool, soaked in water, and the four cultures were placed on the cotton-wool. The dish was exposed to direct sunlight and covered with a large glass bell to prevent evaporation taking place too quickly. The glass bell was raised a few cm., and by means of a glass tube cool water was circulated round the cultures when the temperature rose too high. A maximum and minimum thermometer was placed under the glass bell and a water thermometer immersed in the cotton wool.

Four control cultures, prepared in the same way, were placed in an incubator at 35° C. The maximum temperature of 43° C. under the glass bell was noted at 3 p.m., 25° C. was recorded in the cotton-wool and 32° C. in the open. At 5 p.m. the cultures were placed in an incubator.

28/6/15.—At 7 a.m. to note the influence of sunlight for one day only, two cultures were left in the incubator, whilst a gelatine and a liquid culture were exposed to the sunlight as on the previous day.

29/6/15.—At 7 a.m. on examining the different lots of cultures, the following results were noted :—

Culture exposed for two days to the sunlight.

50 per cent. dead eggs.

50 per cent. dead larvae, of which a few were in the second stage.

Culture exposed for one day to the sunlight.

50 per cent. dead eggs.

50 per cent. larvae in the second stage and were well developed.

Control cultures in incubator.

5 per cent. dead eggs.

95 per cent. larvae in the second stage and were well developed.

On the following day the larvae in the control cultures and the surviving larvae in the culture exposed for a day only had reached maturity.

30/6/15.—A second experiment was undertaken similar to the previous one, but to avoid evaporation from the cultures the glass bell was dispensed with, and from time to time small quantities of water were added, having the same temperature as the culture medium. The day was cloudy in the morning, and the temperature was about 20° C.

1/7/15.—The day was sunny; the cultures were placed in the sun, the maximum temperature in the sun being 40° C. and in the cotton-wool 24° C.

2/7/15.—The cultures exposed during the two previous days contained dead eggs and dead larvae in the first stage.

The control cultures contained living and well developed larvae in the second stage.

A number of other experiments with eggs in agar and liquid cultures were carried out in January, 1915.

The cultures were exposed to the sun at about 7 a.m., and supplied with the necessary amount of water from time to time.

Twice, in cultures started with eggs at the embryo stage, free embryos were found at sunset.

In a third instance the eggs hatched during the first night, and in four other instances the eggs were found to be granular and dead at sunset after the first day of exposure.

In the three instances in which living larvae were found after one day's exposure they were found to be dead after the second day of exposure.

In a series of experiments faeces, of sheep were used as a medium. The droppings were placed on moist, black turf, and sprinkled with water from time to time during the day. The experiment was carried out during different seasons of the year and always proved positive. In the summer time a rather large number of young larvae were found dead in the more superficial layers of the pellets, but the remainder reached maturity in the usual time.

Observations on the above experiments.—The first two experiments made in winter (June) show that eggs exposed to the sunlight for about half a day during winter time, and kept at a suitable temperature, remain alive, but at the end of the second day of exposure the hatched larvae are invariably killed.

The experiments conducted in summer time show that eggs exposed for about a day to sunlight at a temperature kept artificially at normal are sometimes still living at sunset, but more frequently are dead, and the hatched larvae are invariably killed on the second day of exposure.

The action of sunlight on mature larvae.—On the 8/3/15, and on the 7/4/15, at 7 a.m., mature larvae from 10–12 days old were placed in Petri dishes with a layer of water 5 mm. deep. The Petri dishes were put on soaked cotton-wool, as mentioned previously, and were exposed to direct sunlight for a whole day.

They were examined the following day, and only 1 per cent. of the larvae were moving, the remainder being dead. After the second day of exposure no living larvae were found. The days of exposure were bright and warm, but the temperature in the water did not exceed 32° C.

On the 12/5/15, another lot of faeces was exposed under similar conditions as above for a number of days. The result was as follows:—

After the 1st day of exposure	20 per cent. of larvae were dead.
„ 2nd	60 „ „ „
„ 3rd	75 „ „ „
„ 4th	1–2 per cent. were found slightly coiled up, but were not moving about.

The temperature during the day was frequently 46° C. in the sun, but the effect of the sunlight was evidently weaker than in summer time.

The Action of the Sun on the Eggs and Young Larvae naturally exposed in the Field.—Some experiments were carried out with infected faeces placed in the field and exposed to the action of the sun on the bare soil.

(1) On the 4/3/15, at 7 a.m., infected faeces were collected separately from three sheep. A culture was made from each lot of faeces, and the remainder of the pellets were scattered on to black earth and left till sunset. The maximum temperature was 40° C. in the sun. In the evening three cultures were made with the dry faeces. Four days later the control cultures contained numerous larvae, but the three cultures made with the exposed faeces proved negative.

(2) Exposure for part of the day: On the 11/3/15 the faeces were exposed from noon to sunset at a maximum temperature of 53° C. The culture proved negative; the controls were positive.

8/3/15.—The infected faeces were exposed from 7 a.m. to 12 a.m. at a maximum temperature of 53° C. The culture proved to be negative and the control one gave positive results.

(3) Constant exposure during winter time: On the 26/6/15, faeces passed the previous night were placed on black earth at 12 a.m. and exposed to the sun at a temperature of from 35–46° C. A culture was made at sunset and numerous larvae were found within the next three days.

On the following day the temperature was 42° C. in the sun. A culture made from faeces collected at sunset the same day proved negative. Faeces collected from the same lot on the 3rd day at sunset also gave negative results.

A control culture, also exposed to the sun during the same time as the above lot and constantly kept moist, contained well developed larvae in the second stage after the second day of exposure.

Some observations were carried out in the field with infected faeces placed amongst grass.

NOTE.—In the following experiments the atmospheric conditions will not be alluded to, as a daily report for the month of March will be found on page .

(1) Faeces deposited in single pellets after sunset amongst short dry grass, during a period of changeable weather :

23/3/15.—At 6 p.m. faeces were placed in grass 6–7 cm. high, partly protected from the sun. The earth was dry.

25/3/15.—In the evening the pellets were examined and were found to be apparently dry, but no larvae were present. A culture made from pellets the same evening only contained dead eggs.

On the 28th and 29th March and 1st of April, pellets of the same lot moistened by rain were examined, but only contained dead eggs.

It appears that the low temperature of the first night did not allow the eggs to reach the embryo stage, and the sun of the following day killed them.

(2) Experiment with faeces deposited in single pellets in high grass in the morning :

26/3/15.—At 8 a.m. infected faeces were placed amongst grass 20 cm. high, well protected from the direct rays of the sun. The ground was dry.

26/3/15.—The faeces were fairly dry at 6 p.m.

1/4/15.—The pellets had been slightly moistened by heavy dew.

4/4/15.—Very few larvae, poor in granulations, were found.

It appears that the tall grass did not prevent the sun and dry wind on the 26/3/15 from killing nearly all the eggs, and only a very few eggs in the centre of the pellets survived.

(3) Experiment with faeces in single pellets deposited amongst grass of medium height. Moist weather ; cold nights.

28/3/15.—At 8 a.m. the faeces were placed in layers amongst grass 8–10 cm. high.

1/4/15.—On microscopical examination moist and well protected faeces gave the following results :—

100 Larvae had hatched and were dead ;

10 Eggs contained living embryos ;

20 Larvae were in the second stage and living ;

2 Larvae in the second stage were dead ; and

2 Well-developed mature larvae were alive.

The superficial pellets contained 5 per cent. living larvae, the remainder dead eggs. In subsequent observations only 10 per cent. larvae reached maturity in the well protected faeces.

In consulting the daily atmospheric report for March in the above period, it appears that the conditions were quite favourable for the development of larvae, with the exception of the nights, which were rather cold.

(4) Experiment on the exposure of faeces in single pellets deposited in the field in the morning in tall grass. Variable weather.

30/3/15.—At 8 a.m. faeces in pellets were placed well protected amongst grass about 15–20 cm. high.

3/4/15.—95 per cent. of larvae were dead and had reached the first stage; the remaining 5 per cent. were in the beginning of the second stage and were rather poor.

4/5/15.—Very few larvae had reached maturity and were living.

It appears that the first day was favourable for the hatching of the eggs, but the following cold nights did not allow of development; the afternoon of the 30/3/15 was hot and bright, killing the larvae in the first stage. The low temperature of the following nights was not favourable for the development of larvae presumably in the first stage, and the bright days killed a large number of larvae.

(5) Experiment with faeces in single pellets and clustered pellets deposited in the morning amongst tall grass. The nights were cold.

5/5/15.—Two lots of faeces were placed amongst grass 20 cm. high; one lot in single pellets, the other in clusters.

8/5/15.—The single pellets contained eggs with grown embryos, and the more protected faeces in clusters contained a large number of larvae.

15/5/15.—In the single pellets the eggs were still in the same stage, and apparently dead. In the clusters of faeces only dead eggs and a few living larvae in the second stage were present.

During the first days of exposure the sun was bright and the atmosphere was rather dry; the eggs in the single pellets were killed, whilst the pellets in the centre of the clusters, owing to the sun not being very hot, contained sufficient moisture to allow the larvae to reach the end of the second stage. The moisture during the following days was rather scarce, the nights were cold, and it is presumed that the larvae migrated into the ground.

General Observations on the Experiments carried out in the Veld: Constant Warm, Wet Weather.—When the weather is warm and rain is constantly falling for three days, or the ground is kept moist for the same length of time, with a heavy cloudy sky, the majority of larvae reach maturity.

Constant Warm, Dry Weather.—When the weather is warm and dry, as during periods of drought in summer, or dry and sunny as in winter, practically all the eggs and young larvae in scattered pellets are killed in two or three days, even if amongst ordinary veld grass.

Variable Weather.—Concerning variable weather, it is not possible to give any definite rule, owing to the action of the ambient being too complex.

In comparing the results of the above experiments with the condition of the weather at the time, it can be concluded that if the first two days are dry and sunny, a very high percentage of eggs and larvae just hatched

are killed. There seems to be no difference if the faeces are deposited at night, as even if the eggs hatch during the night, the larvae in the first stage, which are particularly delicate, are killed during the following day. If infected faeces are placed on dry soil, numerous eggs die, even if the first day is cloudy. On the other hand, if the faeces are dropped after heavy rain, numerous eggs survive in infected faeces situated in grass, even if the first day is sunny and warm.

In the third and fourth experiments it was noticed that the highest mortality of larvae occurred in those in the first stage, thus confirming the results referred to in the laboratory experiments. Further, it was noticed, particularly in experiment No. 3, that notwithstanding a certain amount of moisture being present, the mortality of larvae can be very high.

In supposing that the mortality referred to in experiment No. 3 is due to cold nights, then a difference in the results between the laboratory and field experiments should be very noticeable. As reported in the laboratory experiment on oscillating temperatures, the faeces exposed daily for six hours at 10° C. contained 70 per cent. mature larvae after three days.

Experiment No. 5 shows that if the faeces are deposited in clusters amongst tall grass, some larvae reach maturity, even if sunny days predominate, providing the temperature is not too high.

When infected faeces are passed out on to the veld, a fall of rain, even if prolonged for a day at a time, is not sufficient to allow the majority of larvae to reach maturity, if sunshine is again constant for a few days. Consequently, during periods of drought in South Africa, the majority of eggs and larvae on the veld die. It is only during protracted rainy weather that a large percentage of eggs reach maturity. This fact, connected with the facilities the mature larvae have of reaching the host if the surroundings are moist, explains the heavy infection that is noted in a flock after a heavy rainy season.

In conclusion, even in summer the percentage of larvae reaching maturity is very low, but the number of eggs deposited in a field by infected sheep is very high, as will be shown later. The sun is the chief agent of destruction for eggs and larvae under the natural conditions of the veld, and the mortality increases with increased dryness of the soil and atmosphere.

To comprehend the opportunities the sheep have of becoming infected, in spite of the high wastage of eggs and larvae, the following observation is of interest:—

Single pellets of faeces collected from an infected sheep were placed in a glass dish on a slide, and kept in a suitable room for the development of the eggs. Two days later, in a pellet weighing 0·800 grammes, 3000 developed larvae were found, the unhatched eggs not being taken into consideration.

During the day on which the faeces for the above culture were obtained, the sheep passed 303 pellets, and during the next night 704 pellets. Calculating now on the basis of a thousand pellets being passed during twenty-four hours, the number of larvae would be 3,000,000.

During the following days the counting was repeated, and the same number of larvae was found.

From the above observations, an approximate idea can be formed as to the amount of infection spread daily by an infected flock.

The Action of the Sun on Mature Larvae: In Faeces.—An observation on the exposure of mature larvae in pellets during winter was recorded in table No. 4, in the paragraph "Dryness." From this observation it appears that after a day of exposure, in which the maximum temperature was 42°C ., in the sun, about 97 per cent. of the larvae died. The following day the maximum temperature was 35°C . in the morning, and 46°C . in the afternoon, when the living larvae were reduced to 1–2 per cent. This minimum was still found after 4, 6, 14, and 25 days of exposure, when during the last three days there were heavy clouds and rain.

Exposure of mature larvae in droppings was also attempted in some instances during summer. The single pellets were directly exposed to the sun on the bare soil. The larvae were found dead after a day of exposure.

In the field mature larvae in faeces survive longer when protected by grass from the direct sunlight.

On Dry Grass.—Experiment No. 1.—In the evening blades of dry grass were placed on the internal walls of a jar culture; migration of larvae to the grass then took place. The following morning at 7 a.m. the same blades were exposed to the sun in an upright position, in such a way that one surface was facing the sun. In the evening both surfaces of each blade were washed in separate glasses. After twenty-four hours it was noted, out of some 400–500 larvae, none were living on the surface exposed to the sun. On the opposite side, however, from 20–30 per cent. living larvae were found. The day of exposure was dry, the maximum temperature being 40°C . in the sun.

Experiment No. 2.—On the 22/8/14 pieces of dry open blades of grass, on to which mature larvae had crawled as in the first experiment, were exposed in a room for three days. On the morning of the 4th day 70 per cent. larvae were still living, and a number of pieces of the same grass were exposed to the sun at 7 a.m., under the same conditions as in the first experiment. Every evening a piece of grass was placed in water and was examined next morning. The result was as follows:—

At the end of the —

1st day,	at a maximum temperature of 36°C .,	20 per cent.	were living.
2nd	"	"	"
3rd	"	"	"
4th	"	"	"

	36°C .,	15	"	"
	39°C .,	5–6	"	"
	40°C .,	3–4	"	"

Other observations were made at the end of the 5th, 6th, and 8th days, when all the larvae were found dead.

Experiment No. 3.—On the 16/4/15 larvae fifteen days old were collected on dry open blades of grass, and exposed to the sun the following day, under similar conditions as in experiment No. 1. The result was as follows:—

At the end of the—

1st day,	at a maximum temperature of 40°C .,	40–50 per cent.	were living.
2nd	"	"	"
3rd	"	"	"

	40°C .,	2	"	"
	42°C .,	all were dead.		

Other observations were made after the 4th, 5th, and 6th days when all larvae were found dead. (The maximum temperature in the sun was 43° C.)

Experiment No. 4.—Larvae were exposed on dry, shrivelled up grass. On the 5/5/15 pieces of green grass, on to which numerous larvae had crawled, were exposed to the sun under the same conditions as in experiment No. 1. The day was dry and windy, with a maximum temperature of 35° C. After three hours the pieces of grass had a dry, shrivelled up appearance. The resistance of larvae was recorded as follows :—

At the end of the—

1st day 20 per cent. of larvae were living.
 2nd day 10 per cent. of larvae were living (maximum temperature 39° C.).
 3rd day 4 per cent. of larvae were living (maximum temperature 39° C.).
 8th day 1-2 per cent. of larvae were living (maximum temperature 41° C.).

Experiment No. 4.—On the 26/3/15 a certain amount of infected faeces was placed at the foot of a dry grass plant in a flower pot, covered with a glass bell, and constantly moistened. On the 9/4/15, early in the morning, a large piece of the dry grass was examined and numerous living larvae were detected. The flower pot was then placed in the sunlight, and the blades of grass commenced coiling up. The results of exposure to the sun during the following days were as follows :—

At the end of the—

1st day	at a maximum temperature of 32° C.,	84 per cent.	were living.
2nd	"	"	"
3rd	"	"	"
4th	"	"	"
5th	"	"	"

43° C., 6 " "
 40° C., 2 " "
 40° C., 1·3 " "
 40° C., all were dead.

Up to the 5th day only blades of grass were examined; on the 8th day two pieces of stalk from the same grass plant were cut and examined, and 60 per cent. larvae were found to be dead. On the 9th and 10th days the examination was repeated in the same way, and 50-60 per cent. were found to be dead.

On Green Grass.—Experiment No. 1.—A large number of young mature larvae were spread at the foot of a grass plant growing in a flower pot. The plant was constantly kept moist, and covered with a glass bell. The flower pot was exposed to diffused light. On the morning of the 11/8/14, when large numbers of larvae were found on the grass blades, the flower pot was suddenly exposed to the sun. The three following days were sunny, with an average maximum temperature of 39°-40° C. in the sun, and a minimum relative humidity of 18 per cent. On the 14/8/14, three days later, at 7 a.m., 60 per cent. of larvae were found dead on the blades of grass. From the 14/8/14 to the 22/8/14 the maximum average temperature in the sun was from 35°-40° C.; the daily minimum humidity was 10 per cent. On the 22/8/14, eleven days later, at 8 a.m., on three open blades of grass, all the larvae were dead. On the 26/8/14, fifteen days later, on three open blades of grass all the larvae were dead. The same result was obtained 17, 21, and 24 days later.

NOTE.—The grass was watered every morning, and during the night was sheltered on the veranda from the dew.

Experiment No. 2.—At the foot of a green grass plant growing in a flower pot, infected faeces were placed, constantly kept moist, and covered with a glass bell. On the 22/3/15, when the mature larvae were migrating on to the grass in large numbers, the flower pot was exposed to the sun. The result of successive observations was as follows:—

22/3/15.—At 6 p.m. 5 per cent. of larvae on blades were dead.

23/3/15.—At 6 p.m. 10 per cent. of larvae on blades were dead.

24/3/15.—At 6 p.m. 10 per cent. of larvae on blades were dead.

26/3/15.—60 per cent. of larvae on blades were dead.

31/3/15.—80 per cent. of larvae on blades were dead.

1/4/15.—All larvae on blades were dead.

2/4/15.—80 per cent. larvae on blades were dead.

5/4/15.—All larvae on blades dead.

6/4/15.—All larvae on blades dead.

7/4/15.—All larvae on blades dead.

8/4/15.—Two stalks with four blades of grass were cut, and examined; 15 per cent. of larvae were dead.

15/4/15.—The examination of stalks was again undertaken and 60 per cent. of larvae were found dead.

30/4/15.—The blades of the grass were dried up, but the stalks were still green. All larvae were dead.

5/5/15.—On the dry blades of grass 10 per cent. of larvae were found living.

7/8/15.—On two dry stalks of grass, twenty-four living larvae and twenty-one dead larvae were found.

(The last rain fell seventeen days previously).

11/9/15.—On two dry stalks of grass, twelve dead larvae were found. (From the date of the previous observation practically no rain fell.)

14/9/15.—On two dry stalks of grass, three living larvae and six dead larvae were found. (A heavy rain fell in the night between the 12th and 13th of the same month.)

NOTES.—In the second experiment the big mortality of larvae was observed later than in the first experiment, but the weather in the second experiment was not as harmful.

In the examination on the 31/3/15, I expected to find all the larvae dead as in the previous experiment, but, in all probability owing to the thunderstorm on the previous day, a new lot of larvae migrated on to the grass from the faeces. In this way can also be explained the reappearance of living larvae on the 2/4/15, 5/5/15, 7/8/15, and 14/9/15, after negative results were obtained. The high percentage of larvae still living on the stalks of grass after repeated negative results on the open blades illustrates the efficacy of shelter against sun exposure, and indicates further that larvae may survive on the stalks after escaping from the sun. The points mentioned in this note will be dealt with again under "Migration of Larvae."

NOTE.—For further details on the atmospheric conditions during the above experiments, see page 420.

Conclusions on the Experiments made with Mature Larvae on the Grass.—

The resistance of the larvae of *Haemonchus contortus* exposed to the sun on dry grass depends on the position of the larvae on the grass and on the condition of the blades, viz., if they are open or shrivelled up. The dry, shrivelled-up grass preserves the larvae for a longer period than the open grass.

From the above observations, it appears that larvae on dry grass in the open veld do not survive for longer than 8–10 days. This period need not, however, be considered to represent the maximum time during which larvae can resist, but, nevertheless, it does not seem probable that the larvae on dry grass are able to resist through a long period of drought in summer or in winter.

On green grass the difference in resistance is comparatively small. A large percentage of larvae die within the first 3–4 days after sun exposure when on the blades. On the stalks the resistance varies within wide limits, according to the nature of the blades and grass stalks. Experiments on a large scale have not so far been made. From experiment No. 2 the period of resistance on the stalks would hardly be a month, although during the time of observation rain fell on several occasions.

It must be noted that in the veld the stalks of grass are frequently collected in a thick tuft, which remains green practically all the year, whilst the plant used in experiment No. 2 had separate stalks. The resistance of the larvae in tufts will receive special attention subsequently.

The experiments with the resistance of the mature larvae on grass are not so important as it was thought before, owing to the fact that during a rainfall a new lot of larvae migrates from the soil to the grass.

MIGRATION OF THE MATURE LARVAE.

Thigmotropism of the Larvae.

During the period of their growth, the larvae are principally engaged in feeding and in finding suitable conditions for their further development.

When the larvae are in a faeces medium, as is the rule in the veld, food is abundant, and the movements of the larvae are chiefly to the outside of the pellets where there is a better supply of air. They return into sheltered layers of the pellets when the conditions of light, moisture, and temperature are not suitable on the surface. This probably occurs when the larvae are undergoing the lethargic stage.

During their growth the larvae show a distinct preference to migrate towards the ground, and this is probably due to the fact that there is frequently more moisture in the lower layers of the pellets and in the pellets touching the ground.

When the larvae reach maturity a new feature appears in their movements, viz., a tendency to leave the medium in which they developed. This fact was observed :—

- (1) In jar cultures with faeces, where the migration of mature larvae on to the walls is usually conspicuous.
- (2) In agar cultures where mature larvae were found on the walls of the Petri dish ; and

- (3) In liquid medium in watch glasses, where mature larvae were found coiled up above the level of the liquid.
- (4) In cultures made at the base of a tuft of grass, when mature larvae are found on the grass blades 4-5 days afterward if the conditions of the ambient are favourable.

The same fact was not observed in liquid medium in Petri dishes, as on the vertical glass walls the larvae do not seem to be able to crawl out of the liquid. When, however, mature larvae are placed in liquid or agar medium they remain there. This is due to the cultures containing toxic substances excreted by the larvae.

The mature larvae migrate from the culture medium in all directions, but they prefer crawling on to supports rising vertically over the medium in which they are. Sometimes on the 3rd day, but more frequently on the 4th day, mature larvae were seen in great numbers crawling up the walls of the jars. The colony could then be clearly recognized by the naked eye as a whitish patch, resembling frost on a window-pane, and such patches could be seen to change their position. The distribution of the colony resembles the branches of a tree, the branches being formed by a continuous line of larvae. This continuity of the larvae and the large number of anastomoses is a constant feature of a moving colony. (Fig. 23A.) When a drop of water was placed on a partially dry colony the larvae were seen to start their movements as soon as the water commenced spreading over the slide following the branches of larvae. It appears, consequently, that the continuity of the larvae forming the ramifications plays an important rôle in the distribution of water for the benefit of the whole colony. This fact appears to be of great importance in the natural migration of larvae when the dew is on the grass. It was observed when larvae were isolated in a drop of water they were not able to leave the drops.

To obtain a rapid migration of larvae on to the walls of the jar, it is advisable to spread the faeces at the foot of the walls of the jar. When the mature larvae are unable to reach the walls of the culture glass, viz., when a heap of faeces is disposed in the middle of a glass dish without contact with the walls, they crawl on to the top of the faeces, and many of them erect their bodies, trying to find a higher support. Sometimes two or three larvae cling together, visible to the naked eye as small white dots. If a stick is fixed perpendicularly into a heap of faeces, numerous larvae will shortly afterwards be found crawling up the stick. The larvae are very persistent in their crawling movements, and I attempted to stop the migration by spreading vaseline along the walls of the jar, but if the colony is a strong one the larvae pass through it very easily. If the jar is closed with a slide cover, the larvae soon find if there is an outlet and then escape. If the conditions are favourable, the larvae travel further and spread on to the outer surface of the jar.

For diagnostic purposes in working with cultures, when a number of test-tubes were corked with plugs of cotton wool and placed in a large specimen jar, it was observed that after a lapse of some days larvae were frequently present on the walls of the jar, having escaped from different test tubes. If the escaped larvae find the external ambient too dry, they collect together along the commissure between the cover and the jar, and then form one or more dark yellowish clusters, some of which are as large

as a maize grain. If such a cluster is immersed in water, the latter becomes turbid with swimming larvae.

The mechanism and velocity of the travelling larvae vary slightly, according to the supporting surface and the influence of external agents.

Agar Plates.—The most efficient support for the movements of the larvae is semi-solid medium, or slightly moistened rough solid surfaces such as a grass blade. The travelling of the larvae, observed on the surface of an agar plate, can be compared with that of a snake.

At 15° C., with the usual reflected light under the binocular microscope, on an agar plate (solution of 1 per cent.) young mature larvae crossed the field of the microscope (3 mm. in diameter) in 8 to 12 seconds. Within the agar jelly (1 per cent. solution) larvae were found travelling with great facility, and presented the same features in their movements as on the surface. They crossed the field of the microscope (3 mm.) in 12 to 15 seconds. The larvae freely passed from the surface into the mass of the gelatine, and vice versa. In agar plates, in which flakes of cotton-wool were embedded, the larvae passed through the flakes with the same speed as through the homogeneous medium.

Faeces in Pellets.—A suitable method for observing the movements of the larvae on faeces, is to place pellets on a glass slide in a moist glass dish exposed to a diffused light. If on the following days the slide is transferred under the binocular microscope, in the same diffused light, and examined with a dark field, numerous larvae will be seen wandering on the surface of the pellets. The immature larvae are very slow, moving the head only. The mature larvae are quicker, but not as fast as on agar, probably owing to the fact that in a dark field the larvae are not disturbed by concentrated light.

Grass.—On moist grass blades, the mature larvae have the same velocity as on the agar plates.

Glass.—On the moist surface of a glass slide, the movements of the larvae are distinctly more of a wriggling nature than on agar plates, and the progress of the larvae is not so fast and easy, owing to the fact that the glass surface does not offer as much support as the agar.

Fluid Medium.—In water the movements of the larvae are of a wriggling nature, and their progress in a horizontal direction is slower and more difficult than on the glass surface.

Larvae from the same cultures as used for the observations on agar, and in the same external ambient, were placed in a layer of water 2 mm. deep. The larvae took 35–40 seconds to cover the distance of 3 mm. of the field of the binocular microscope.

If a watch glass, in which larvae are immersed in a thin layer of water is placed under the binocular microscope, a number of them attempt vertical movements. As a result of vigorous wriggling movements, the larvae succeed in climbing for a distance equal to once or twice the length of their bodies, but they fall to the bottom again in spite of all efforts. These attempts can be observed for a day or two in the same lot of larvae, but if the conditions of light and temperature do not change, the larvae finally remain at the bottom, and only move slightly.

When larvae are placed in deep water they remain suspended for rather a long time before falling to the bottom. In a large graduated tube, containing a column of water 20 cm. high, mature larvae were placed at the bottom by means of a long thin pipette. When removing the pipette, I noticed that the last few larvae to be ejected remained suspended in the water for at least ten minutes. The tube was left motionless until all the larvae were at the bottom, and then was gently placed in the sun, near a window. After a short time several larvae were seen moving and rising through the water to the top, and then slowly descending again. When the sun disappeared the ascending movements of the larvae were no longer noticed. The following morning the tube was exposed to the sun for about two hours, during which period some larvae were again observed ascending and descending, taking about $1\frac{1}{2}$ minutes to ascend, and the same to descend. When the tube was again placed in diffused light, no larvae were observed travelling through the water. After 11–12 days no larvae were observed to be ascending, even when the tube was exposed to sunlight. At this time the larvae were examined, and half were found to be dead, and half were dormant and coiled up, but showing signs of life. Consequently it may be concluded that mature larvae are unable to rise in still water, but can do so when vertical currents occur in the mass of the water.

On the other hand, the ascent and descent of larvae is due not only to changes in the density of the water, but to their activity as well. This view is supported by the fact that in the above experiment no motionless larvae were seen to be carried by the water.

Influence of External Conditions on the Migration of Larvae.—Moisture.—Larvae remain motionless unless a certain amount of moisture is present. In a jar culture the larvae will not crawl on to the wall after it has been dried with cotton wool.

Similarly, to induce migration of mature larvae along the blades of grass from infected faeces placed at the foot of the grass kept in a flower pot in a room or on a veranda, it was necessary to cover the plant with a glass bell to preserve the moisture. If larvae in migration are overtaken by dryness, they collect together, or simply coil up. Larvae that were drying up on a slide were seen to collect on the spots where the moisture remained longest. In an experiment undertaken for another purpose on 31/3/15, it was noted that larvae on the walls of a jar retired for a distance of 10–11 cm. towards the faeces after sun exposure. The jar was opened twenty minutes later, and the larvae were observed to have retired further back to the level of, and partly into, the faeces. Some other larvae, apparently too far away when overtaken by dryness, were coiled up and remained on the same spot. This phenomenon was repeatedly observed in jar cultures when the lid was purposely removed to observe the effect of dryness on the migration of the larvae.

Another observation on the same subject is the following:—It frequently happened in jar cultures kept in a room, that the deposit of moisture on the inner walls disappeared from one place and reappeared on another spot. If the larvae had already commenced their ascendant migration, it could be clearly seen that they followed the moist places.

Temperature.—A warm ambient is favourable for the migration of larvae. A temperature of 40°–42° C. appears to be the highest point at which the larvae remain active for any considerable length of time. At higher temperatures the larvae still move, but only for a short time, as they soon get exhausted and motionless. Low temperatures retard the migration of larvae. Diagrams drawn from jar cultures kept at 9°–10° C. show that the migration is not stopped, but the speed is reduced.

(1) 18/4/15.—Larvae in a culture in faeces constantly kept in an incubator at 35° C. for four days, had migrated 7 cm. above the level of the faeces.

On the walls of the vessel containing the culture, a line was drawn marking the upper limit of the area covered by the migrating larvae.

The curve shown in Chart No. 1—A is a reproduction of this line, showing the height and form of this upper limit.

It may be stated that during the further course of this paper the word "curve" will frequently occur, and is to be understood as being used in the same sense.

When placed in direct sunlight they descended 2–4 cm. (Chart No. 1—B). The culture was then placed in the cold storage at 9°–10° C. for twenty-four hours. On the 19/4/15 the larvae had descended a further 1–2 cm., appearing in white patches, and had risen in some places 4 cm. above the previous level (Chart No. 1—C).

Placed in the direct sunlight, no alteration was noted in the curve. The culture was then placed in a dark room at 10 p.m., at a temperature of 18°–20° C. At 10 p.m. the larvae rose uniformly around the jar for a distance of 4–10 cm. (Chart No. 1—D. Chart No. 2—A).

20/4/15.—Another exposure to the sunlight caused the larvae to descend 11 cm. (Chart No. 2—B). In the evening the culture was replaced in the cold storage.

21/4/15.—A rise of the colony of larvae was noted all along the curve to a maximum of 7–8 cm. (Chart No. 2—C).

Concerning migration on the grass, it has been mentioned in experiment No. 5 (Temperature in the Field) that on the morning of the 8/7/15, after an all-night rain and a minimum temperature of 10° C., living larvae were found moving on to the grass.

(2) On the 26/7/15, at 10 p.m., a jar culture 11 days old showed a strong migration of larvae over a large area, 10 cm. high, the ambient temperature being 15° C. The jar was placed in the open when the temperature was about 1° C. During the night the minimum temperature was –3° C. At sunrise the following morning the temperature was 5° C., but the larvae were still at the same level as the previous evening, and had collected in small oval patches.

On the 29/7/15, at 6 p.m., the jar was placed in the open; the larvae had formed numerous branches rising 8–9 cm. above the level of the faeces. The temperature was 15° C. At 10 p.m. the temperature was 5° C., but no further progress was noted. During the night the minimum temperature was 1° C., and in the morning it was noticed that the larvae had not changed their level, but the larvae in numerous branches of the previous evening had collected into thick oval patches. The following night at 10 p.m., the temperature of the room being 18° C., it

was observed that the same colony of larvae had reached a height of 16–18 cm. above the level of the faeces. It is therefore evident that the larvae stopped their migration when the temperature decreased from 15° to 5° C., and collected together in clusters, remaining at the same level. The conclusion is that larvae discontinue their ascendant movements with a decrease in temperature and when a temperature of 16°–5° C. is reached. With a further decrease in temperature the larvae do not descend, but collect in patches and remain motionless until the temperature rises again.

Phototropism of Mature Larvae.

Night time appears to be favourable for the migration of larvae. When a jar culture was exposed in the open, the highest migratory curve was noticed from 9–10 p.m. till sunrise.

If a culture is kept in constant darkness at the optimum of temperature, the migration begins at the usual time, but the progress of the colony is slow, and often the maximum height is not reached. If the culture is exposed to a diffused or direct sunlight for some hours and then placed back in darkness, an extensive and quick migration of larvae could be noticed.

Diffused Light.—A weak diffused light, observed in heavy cloudy weather, is also favourable for the migration of larvae. They show a gradual progress by day and night. It was also observed that larvae in a dark place were attracted by diffused weak sunlight. In this connection the following facts were observed :—

- (1) If a culture of larvae in the migratory period was placed in a room with bright diffused light, it was noted that from sunset to sunrise the larvae prefer migrating on to the wall of the jar opposite to that facing the window.
- (2) If a culture of larvae in migration is placed on to a window table in heavy cloudy weather, or in the night, the larvae collect on the wall facing the window.
- (3) If a culture of migrating larvae is placed in a cupboard with the door left partly open, the larvae collect on the wall facing the opening.

It was also noticed that larvae are attracted from the darkness towards a weak artificial light. An experiment was carried out in the following way :—

A jar culture, with mature larvae uniformly distributed over the walls, was placed in a closed box having a narrow opening at one end. In front of the opening a gas-lamp was placed at a distance of 50 cm. The room in which the experiment was made was nearly dark. At the end of an hour the majority of larvae had collected in thick patches on the illuminated area.

From the above observation it can be concluded that larvae of *Haemonchus contortus* are positively phototropic. In comparing the same observations with those on “Bright diffused light,” it must be concluded that they possess a limited positive phototropism. They are, in other words, seeking for a suitable intensity of light.

The intensity of light most suitable for the larvae of *Haemonchus contortus* is that observed in the open in rainy weather.

Experiments to Show the Behaviour of the Larvae on the Walls of a Jar placed in a Room in Ordinary Diffused Light.—In a jar culture exposed in the laboratory small branches of larvae were observed on the 4th day spreading from the level of the faeces; by the afternoon some branches were reduced to small patches at the level of the faeces, whilst others had disappeared. On the 5th day, at daybreak, the branches of larvae were 2–5 cm. high; by the afternoon the larvae were observed in patches, sometimes connected with each other by small branches, whilst other larvae were still scattered. On the 6th day, at daybreak, the larvae had risen 6–8 cm. above the faeces; by the afternoon the colony had descended some distance. On the 7th day, at daybreak, the larvae were in full ascendant migration, some reaching the edges of the jar. In the following days the migratory curve showed an oscillation in direct proportion to the intensity of the light until the 20th day, when migration stopped, and the larvae remained dormant on the walls. The above observations were noted in March, 1915, at a temperature of 15°–25° C. The difference in the migratory curve during day and night was constantly observed in numerous other cultures placed under similar conditions.

It was also noted that a culture constantly kept in darkness some days after the larvae had reached maturity, and then exposed to the open by day and night, did not show at once the same degree of nocturnal ascending migration as that observed when a culture was exposed to the same conditions from the commencement. It appears that a colony of mature larvae want some days of training before they develop constant oscillatory movements day and night.

Bright Diffused Light.—A bright diffused light, as observed on a bright day, is decidedly unfavourable for the ascending movement of larvae, and produces a marked decrease in the height of the migratory curve of the previous night. Chart No. 3, Curve A, shows the level reached by a colony of young larvae on the night of the 28/7/15, with a minimum temperature of 5° C., and curve B the level of the colony at 2 p.m. on the 29/7/15 in a bright diffused light at a temperature of 20° C., when they had descended about 7 cm. Where the curves A and B are closer together, it must be inferred that the larvae did not descend owing to their being overtaken by dryness.

On Chart No. 5 it can be seen that a culture which was placed on a window table on the 30/3/15, containing larvae on the wall facing the window (Chart No. 5—A, two distal portions of the curve), the larvae migrated on to the opposite wall (Chart No. 5—B, medial portion of the curve).

Generally the descending migration of larvae during the day was always observed when a culture was exposed on a veranda during bright weather (see Chart 5–17).

Bright Artificial Light.—If a jar culture showing heavy migration of larvae on the walls is exposed facing a gas or electric lamp at a distance of 30–40 cm., the larvae migrate on to the opposite wall. An hour later

usually only a few larvae can be observed on the illuminated wall, and thick tracts are directed in the opposite direction to the light.

Changes in Diffused Light.—During their migratory movements the larvae are highly sensible to variations of diffused light.

A colony of larvae having reached a certain level while the culture is exposed in a room quickly descends to a lower curve if the culture is transferred to the diffused light of a veranda, and sometimes the larvae collect in patches. Vice versa the migratory curve rises at once if a colony is transferred to a room from the verandah in the day time, and the patches of collected larvae spread at once. Chart No. 3 illustrates the above observation. When the migration traced in curve B was obtained by exposure on the veranda, the culture was transferred to a room with a dim light. Within five minutes at a temperature of 17° C., a marked rise in the curve of the colony was observed (Chart No. 3—C).

If a bright day suddenly becomes cloudy, or rain falls, the ascending migration of larvae in jar cultures is immediately observed. In this connection the following experiments were undertaken:—

20/3/15.—A jar culture, made on the 14/4/15, had, during the previous night, been kept in a room at a temperature of 20° C.

On the following morning a large colony of larvae was seen on the walls of the jar 10–14 cm. above the level of the faeces (Chart No. 4—A). The jar was then exposed to a diffused light on the veranda. After a short time, just as the larvae were commencing to descend, heavy clouds gathered, and it remained cloudy until 11 a.m. The culture was then examined, and it was observed that the larvae had ascended 4–8 cm. from the spot where they were in the morning (Chart No. 4—B) (1).

The rest of the day was sunny. At 2 p.m. the culture was again examined and the larvae were found 10–11 cm. lower (Chart No. 4—C).

As a control, the culture was placed in a diffused light on the veranda the following morning, when the curve of the ascending migration was rather high; four hours later the larvae had descended 12 cm.

From the above observations it can be concluded that in sunny weather larvae descend from the higher to the lower parts of the grass, seeking shelter not only from the sun, as will be seen in the following chapter, but also from bright diffused light, always provided that moisture conditions are favourable.

Heliotropism of Mature Larvae.

On this subject a number of experiments were carried out by exposing mature larvae in jar cultures of varying ages to direct sunlight and when the larvae were either in ascending or descending migration.

Some cultures were exposed daily and the migratory curve recorded, of which the following is a typical one:—

On the 26/3/15 a jar culture was made with heavily infected faeces and placed in an incubator at a temperature of 29° C. On the 29/3/15 a small colony of larvae was observed on a wall, forming a curve 1–2 cm. above the faeces. The following day at 9 a.m. the colony had formed a curve 3–6 cm. above the level of the faeces (see Chart No. 5—A), and the culture was then placed on a window table. The wall on

which the larvae were crawling was facing the light. At 5 p.m. tracts of larvae were running towards the opposite wall where a large colony was observed 7-8 cm. above the level of the faeces (Chart No. 5-B). On the 31/3/15 at 7 a.m. the curve of the colony was 7-8 cm. higher than on the previous evening, reaching the edge of the jar (Chart No. 5-C). At 10 a.m. the curve of the colony was 1-4 cm. lower. Thick tracts of larvae were seen converging toward the faeces (Chart No. 5-D). The jar was then exposed to the direct sunlight. At 10 a.m., the colony had descended 10-11 cm. and consisted of a dense collection of larvae 2 cm. above the level of the faeces (Chart No. 5-E). The walls of the jar were still moist, and the jar was then left uncovered. Twenty minutes later the majority of larvae had collected together in thick round patches adhering to the mass of faeces. Scattered larvae were seen 2-3 cm. higher, but were dried up. At 3 p.m. the majority of larvae had disappeared into the faeces; only a few were scattered 1 to 2 cm. above the level of the faeces (Chart No. 6-A). Water was then poured into the faeces and the jar was covered again. The sun shone on the culture till 5.30 p.m. and no migration of larvae was detected, but half an hour later the culture was in the shade and a new migration of larvae was noted 3-6 cm. above the level of the faeces (Chart No. 6-B). At 9 p.m. the curve of the colony was 9-12 cm. above the level of the faeces (Chart No. 6-C). On the 1/4/15 at sunrise (6 a.m.) the curve of the larvae was 12-14 cm. above the level of the faeces at the top of the wall (Chart No. 6-D). The jar was then exposed to the sun. At 8 a.m. the larvae had descended 12-14 cm., and were collected in thick patches on a level with the faeces (Chart No. 6-E). A few larvae formed a curve 1-2 cm. above the level of the faeces, and were stretched out motionless. On examining them under the microscope they were observed to be moving slowly, and the chyle intestinal cells were rich in granulations.

During the five minutes that the jar remained in the room for the drawing of the curve new branches of larvae were observed 1-2 cm. above the level of the faeces. On the 2/4/15 at sunrise the curve of the larvae had reached a height of 7-12 cm. (Chart No. 7-B). The ascending curve was observed to be lower than that of the previous night. Under the supposition that the direct exposure to the sun the previous day had injured the larvae, the jar culture was exposed on the veranda in a bright diffused light. At 3 p.m. the larvae had not reached the level of the faeces as in the previous day (Chart No. 7-C). On the 3/4/15 at 6 a.m. the curve of larvae had reached a height of 14 cm. at two points (Chart No. 8-B). Samples of the faeces in the jar were examined and a fair number of larvae was noticed; 30 per cent. had reached maturity, were rich in granulations, and had not commenced migrating; 70 per cent. were still in the second stage. On the 4/4/15 at 6 a.m. the highest points in the curve were 12 cm. above the level of the faeces (Chart No. 9-B). At 10 a.m. the larvae were descending on the wall facing the light. A large number of larvae had turned towards the opposite wall, slightly ascending (Chart No. 9-C, right half of the curve). The oscillation in the migratory curve from the 5/4/15 to the 15/4/15 is shown in the charts from No. 10 to No. 16. In these charts the curve B represents the level reached by the larvae at 6 a.m., and the curve C represents the

level reached in the previous day. It appears that the difference in the ascent and descent of the curve decreases gradually, but constantly remains above the level of the faeces. On the 15/4/15 at 6 a.m. samples of faeces were examined and numbers of mature larvae, rich in granulations, were found, which apparently had not yet started their migration. The jar was again exposed to direct sunlight. Four hours later the curve had descended 8 cm. on the wall facing the sun, many of the larvae were seen re-entering the faeces, and on the opposite wall they ascended to a height of 4 cm. (Chart No. 16—C). On the 16/4/15 at 6 a.m. the larvae were still at the same level as on the previous day. On examining some under the microscope, the granulations of the intestinal cells were very much reduced, and were slightly yellowish. Some of the larvae were dead, and numerous living larvae without the outer skin were found.

A control culture from the same lot of faeces, dated 2/4/15, and kept in darkness, showed the larvae on the walls of the jar richer in granulations than the larvae in the above culture; practically none of them were without the outer skin. At 3 p.m. the curve of larvae was unchanged, but they had collected in small thick patches and were connected with each other by thin filaments. The larvae in the patches were stretched. On the 18/4/15 the larvae were practically in the same position. Samples of faeces in the higher layers in good condition were examined and numerous living larvae were found coiled up. Faeces from the bottom layers of the jar, moist and decomposed, contained only dead larvae. The medium was then carefully removed without disturbing the larvae on the walls, and black moist earth was substituted. On the 19/4/15 at 6 a.m., the larvae were practically at the same level as the previous day (Chart No. 17—A). The jar was then exposed to the sun for two hours, when the larvae were still practically in the same position, but were collected in thick patches touching the earth (Chart No. 17—B). On the 20/4/15, the curve was unchanged, with the exception of a few isolated branches (Chart No. 17—C). On the 24/4/15 60 per cent. of larvae had migrated into the earth. The jar was exposed to the sun, and after two hours only two or three small patches of larvae were present above the level of the earth; higher up only a few dead larvae were found; the remainder had disappeared into the earth. (Chart No. 18.)

Observations on the above experiment.—The migratory curves recorded during the first two days of exposure at the window confirm the negative phototropism of larvae to bright diffused light. The curves traced during the following days show the decided and marked negative heliotropism of the larvae, and the positive thigmotropism during the night. The curve traced at 6 p.m. on the 31/3/15 shows that the larvae commence their ascending movements immediately after sunset, and by 9 p.m. the curve is nearly at the usual maximum height reached at night. This was also frequently observed in other cultures.

The curves traced daily at sunrise show that the larvae remain at the maximum height until the sun, or a bright diffused light, reaches them.

The successive charts obtained in the above observations show that the mature larvae gradually acquire the maximal migratory instinct in the course of a few days, as the night and day migratory curves are then most widely separated. From this time onwards the oscillations of both curves gradually decrease in amplitude, until the surviving larvae descend into the ground.

The observation that the larvae did not seek shelter in the faeces from a diffused light on the veranda, but only from direct exposure to the sun, again shows that the larvae have a strong reluctance to re-enter the medium, probably owing to the decomposition of the faeces, but they do so when forced.

The observation of the 19/4/15 and following days shows that the larvae prefer to shelter in the earth, as after a certain period of migration they retire into the ground.

The possibility that the migration of larvae might be due to their efforts to evade the drying out effect of the rays of the sun was taken into consideration, but this view cannot be upheld, as it was frequently observed that the walls of a jar, from which the larvae retired, sometimes remained moist for an unlimited period.

In the experiments undertaken on the heliotropism of the larvae of *Haemonchus contortus*, it was found that the best results were obtained:—

- (1) By using jar cultures in which the larvae had just reached maturity.
- (2) By replacing the faeces medium with earth, and this should be done when the larvae are at the maximum height of their migration.
- (3) By exposing the jar culture to the sun at sunrise, or during the early morning, and at sunset when the sun is not strong enough to injure the larvae.

Aberrations in the migratory instinct.—If a jar culture which has been constantly kept in darkness, and in which the larvae are creeping on the walls, is placed in a strong diffused light, or exposed to the direct sunlight, it is observed that the larvae, instead of descending, ascend a few cm. further. This ascending movement, however, does not last long, and the larvae soon commence descending as usual. This fact can be explained by the sudden change of light, with which the larvae are not yet familiar, and as a result they become stupified. In fact, the new curve does not present the fine branches usually observed, and the larvae are scattered in quite an unusual way.

Heliotropism of the Larvae tested on Agar.—Test tubes were filled with an agar medium (5 per cent. solution) 10 cm. high, and allowed to harden. Mature larvae were then placed in the tubes on the surface of the agar jelly, and the tubes were immersed in sand to the level of the jelly and exposed to sunlight or to darkness.

A number of experiments were carried out on the above lines, of which the following is a typical one:—On the 24/4/15 four agar tubes with mature larvae were prepared as mentioned above. Two of them were placed in the sun at a temperature of 35°–40° C., and the other two in a dark cupboard. Two hours later the two exposed to the sun were examined,

and very few larvae were found coiled up on the walls of the tubes 0.5 to 1 cm. above the level of the jelly. 50 per cent. of the remainder were coiled up, or moving in a well defined zone 2 cm. below the level of the jelly; the other 50 per cent. were at the bottom of the test tube, 10 cm. below the level of the medium.

The two test tubes were afterwards placed in a vertical position outside the sand in a weak diffused light. Five minutes later numerous larvae were seen rising from the zone, 2 cm. below the level of the gelatine. Fifteen minutes later a fairly large patch of larvae were visible to the naked eye, actively moving on the walls of the tubes. The tubes were then immersed in sand as mentioned above, and exposed to the sunlight. Within a very short time the colony of larvae on the walls had retired into the medium, leaving a few slow moving or motionless larvae on the walls. At 5 p.m. no other larvae had crawled on to the walls of the tubes. Four days later at 4 p.m., the two tubes were again examined, when some dead larvae were found on the walls of the tubes; the living larvae were in the jelly. When the culture was placed in a weak diffused light, twenty minutes later a migration of larvae on to the walls, similar to that noticed on the 24th, was observed. The two tubes constantly kept in darkness showed half the larvae on the walls coiled up and alive; the other half was found alive at the bottom of the medium.

Geotropism of Mature Larvae.

Experiment No. 1.—On the 1/9/14 water containing a large number of mature larvae was poured into the soil at the foot of a green tuft of grass growing in a flower-pot; the grass was covered with a glass bell and exposed in the open, in the shade. Ten days later the glass bell was removed, and the pot was exposed to the sun. On the 26/9/14, 12 dead larvae were found on three blades of grass. In a $\frac{1}{2}$ c.c. of earth, taken from under the grass at a depth of 5 cm., 8 living larvae of *Haemonchus contortus* were found. On the 5/10/14, 6 dead larvae were found on the dry grass blades. In a $\frac{1}{2}$ c.c. of earth taken from under the grass at a depth of 10 cm., 5 larvae were found, 4 of which were living. The earth was still moist enough to stick to the spoon used for the operation. On the 3/3/15, at a depth of 5 cm., living larvae of *Haemonchus contortus* were found. On the dry grass only dead larvae were found.

Experiment No. 2.—On the 27/8/14 a culture of infected faeces was made at the foot of a green tuft of grass growing in a flower-pot. The culture was covered with a glass bell and kept moist. After a few days the culture was left uncovered in the open. On the 3/3/15 dead larvae were found on the blades of dry grass. A few pellets of faeces were present, and scattered in the flower-pot on the surface of the ground, contained dead larvae. The ground at a depth of 5 cm. contained a few living larvae of *Haemonchus contortus*.

Experiment No. 3.—On the 28/3/15 a flower-pot was filled up with black ground, and sterilized in an autoclave. Two plants of green grass were thoroughly washed and planted in the flower-pot. A culture of infected faeces was made at the foot of the grass and covered with a glass bell. The flower-pot was constantly kept on the veranda and watered

with sufficient frequency to keep the ground moist. On the 16/4/15 numerous living larvae were found on the green grass. In a $\frac{1}{2}$ c.c. of earth, taken at a depth of 4 cm., 25 living larvae of *Haemonchus contortus* were found.

With the object of finding out if larvae are transferred through the ground by watering, the following experiments were carried out:—

Experiment No. 4.—On the 29/9/14, a flower-pot was filled up with black turf and sterilized. A layer of infected moist faeces was then disposed on the surface of the ground. The pot was immersed in water 5 cm. deep contained in a glass dish, and left on the veranda; the water was kept constantly at about the same level, and no water was poured on to the ground in the flower pot. On the 15/11/14, at a depth of 3 cm. living larvae of *Haemonchus contortus* were found. On the 20/11/14, at a depth of 5 cm., living larvae of *Haemonchus contortus* were found.

Experiment No. 5.—On the 5/7/15 a culture of infected faeces was prepared in a flower-pot under a tuft of grass, as described in the third experiment. The flower-pot was partially immersed in water as described in the fourth experiment. The pot was kept in a room with an average temperature of 11°–15° C., but without any water being poured on the surface of the contained soil. On the 11/7/15 it was placed on the veranda in the sunlight, without the glass bell and without the dish of water underneath. The temperature was frequently at freezing point during the night. On the 20/7/15, on the green grass the larvae were rather numerous, the majority being alive. In the superficial pellets of faeces, nearly all larvae were in the advanced second stage. The pellets in contact with the ground contained twice as many larvae as the superficial pellets, the larvae also being in the advanced second stage. In 1 c.c. of ground at a depth of 2 cm., 50 mature larvae were found. The ground still contained a good amount of moisture. The pot was again immersed in water, placed in the room, and the culture was covered with a glass bell. On the 7/8/15 fairly numerous larvae were found at a depth of 8 cm. Five days later samples of earth were taken at a depth of 10 and 15 cms. respectively, when a fair number of living larvae of *Haemonchus contortus* was found in all samples.

Experiment No. 6.—In the second experiment reported in connection with "Sun Exposure of Larvae on Green Grass," it was mentioned that on the 7/8/15, 4½ months after the larvae had reached maturity, 24 living and 21 dead larvae were found on two dry stalks of grass. Presumably these larvae had crawled on to the grass seventeen days previously, during the last rainfall. On the same day a sample of ground from the same flower-pot was taken at a depth of 4 cm. and examined, and in $\frac{1}{2}$ c.c. ground 10 living larvae were found with the outer skin, and still containing a good store of granulations. Living larvae were also found in ground taken at a depth of 2 cm. The ground was half black turf and half sand, and at the time was dry.

The fact that all the larvae were found alive in the ground, whilst on the grass half of the larvae, which had been exposed for only seventeen days, were dead, proves that larvae resisted better in the ground than on the grass.

Conclusions.—From the above observations on the migration of larvae it can be concluded, that—

- (1) The mature larvae of *Haemonchus contortus* crawl on to the grass under favourable conditions of moisture, light, and temperature.
- (2) The larvae withdraw to the lower part of the grass, or into the ground, when the surrounding conditions are unfavourable, and reappear on the grass with the return of favourable conditions.
- (3) The succession of night and day produces a nocturnal ascent, and a diurnal descent of larvae on the grass, provided the conditions of moisture are favourable.
- (4) The period during which a colony of larvae, when not interrupted, performs the ascending and descending migration, was found under artificial conditions to last from 20–30 days. It varies within rather wide limits according to a difference of ambient.
- (5) In the field at the end of the migratory period part of the larvae are found dead or alive on the grass; the majority are found sheltered in the tuft of grass, or at various depths in the ground.
- (6) It was repeatedly observed that the larvae in the ground were the richest in food granulations, and in a better condition of preservation than those on the grass. It appears, consequently, that the “migratory period” results in a natural selection of the species, by which weak specimens will soon die by exposure, and the stronger are able to find shelter and to resist exhaustion.
- (7) The mature larvae stored in the ground are able to pass the winter season without heavy mortality.
- (8) The presence of larvae in the soil cannot be explained by the penetration of the superficial water alone, but is due to “geotropism of the larvae.”

The larvae in the ground represent a reservoir, from which a new migration on to the grass is made under suitable conditions.

Observations on the Weather.

March, 1915.

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|-------|---|
| 22nd | Bright; maximum temperature in the sun, 40° C. |
| 23rd. | Bright; maximum temperature in the sun, 42° C. |
| 24th. | Bright; maximum temperature in the sun, 45° C. |
| 25th. | Cloudy; maximum temperature in the open, 30° C |
| 26th. | Bright in the afternoon; dry wind; maximum temperature in the sun, 37° C.; <i>night</i> , minimum temperature, 12·5° C. |
| 27th. | Dull; maximum temperature, 26° C.; <i>night</i> , cloudy, damp. |
| 28th. | Dull, damp; sharp thunderstorm; maximum temperature, 24° C.; <i>night</i> , rain; minimum temperature, 13° C. |
| 29th. | Dull, shower; maximum temperature, 25° C.; <i>night</i> , rain; minimum temperature, 14° C. |
| 30th. | Dull, sharp thunderstorm; maximum temperature, 25° C.; <i>night</i> , cloudy; deposit from mist. |
| 31st. | Cloudy and bright weather; temperature, 26° C.; <i>night</i> , heavy dew; minimum temperature, 12·5° C. |

April, 1915.

- 1st. Alternately bright and cloudy weather; maximum temperature in the sun, 40° C.; *night*, heavy dew; minimum temperature, 12.3° C.
- 2nd. Bright in the morning; maximum temperature in the sun, 39° C.; shower in the afternoon; *night*, minimum temperature, 12.5° C.
- 3rd. Alternately bright and cloudy; *night*, heavy dew; minimum temperature, 12.3° C.
- 4th. Bright, warm, genial day; maximum temperature, 50° C.; *night*, heavy dew; minimum temperature, 12.5° C.
- 5th. Bright, warm, moderate wind; *night*, minimum temperature, 12° C.
- 6th. Bright, dry; maximum temperature in the sun, 41° C.; *night*, dry, slight wind; minimum temperature, 7° C.
- 7th. Bright, dry; maximum temperature in the sun, 39° C.; *night*, damp, shower; minimum temperature, 10° C.
- 8th. Cloudy in the morning; bright in the afternoon; maximum temperature in the sun, 30° C.; *night*, minimum temperature, 10° C.
- 9th. Bright, dry; maximum temperature in the sun, 32° C.; *night*, minimum temperature, 11° C.
- 10th. Bright, dry; maximum temperature in the sun, 43° C.
- 11th. Bright, dry; maximum temperature in the sun, 40° C.
- 12th. } Bright, dry; maximum temperature in the sun, 38° – 40° C; *night*,
- 13th. } minimum temperature, 11° – 12° C.
- 14th. }
- 15th. Bright, dry; maximum temperature in the sun, 40° C.; *night*, minimum temperature, 8.5° C.
- 16th. Bright, dry; maximum temperature in the sun, 42° C.; *night*, minimum temperature, 8.5° C.
- 17th. Bright, dry; maximum temperature of the air, 26° C.; *night*, dry; minimum temperature, 8° C.
- 18th. } Bright, dry; maximum temperature in the sun, 40° C; maximum
- 19th. } temperature of the air 26° C.; *night*, minimum temperature,
- 20th. } 8.5° C.
- 20th. Dull; damp in the morning; bright in the afternoon; maximum temperature of the air, 25° C.; *night*, minimum temperature, 8.5° C.
- 21st. } Bright, dry; maximum temperature in the sun, 40° C.; *night*,
- 22nd. } minimum temperature, 8.9° C.
- 23rd. }
- 24th. } Bright; dry strong wind; maximum temperature in the sun,
- 25th. } 40° C.
- 26th. } Bright; dry strong wind; maximum temperature in the sun,
- 27th. } 26° C.; *night*, minimum temperature, 4° – 6° C.
- 28th. }
- 29th. } Bright, dry; maximum temperature in the sun, 35° C.; maximum
- 30th. } temperature of the air, 22° C.

May, 1915.

- 1st. Bright; maximum temperature in the sun, 35° C.; maximum temperature of the room, 21° C.; *night*, rainy; maximum temperature of the room, 19° C.
- 2nd. Dull; maximum temperature of the room, 20° C.
- 3rd. } Bright; maximum temperature in the sun, 30° – 35° C.; maximum
- 4th. } temperature of the room, 19° C.; *night*, minimum tempera-
- 5th. } ture, 7° C.
- 6th. } Bright; dry wind; maximum temperature in the sun, 39° – 41° C.;
- 7th. } maximum temperature of the air, 19° C.
- 8th. }
- 9th. Bright; dry wind; maximum temperature in the sun, 35° C.; maximum temperature of the air, 19° C.; *night*, frost, minimum temperature, -2° C.
- 10th. } Bright; maximum temperature in the sun, 38° – 40° C.; maximum
- 11th. } temperature of the room, 18° – 20° C.; *night*, minimum tem-
- 12th. } perature, -2° to -3° C.
- 21st. }
- 22nd. } Night, minimum temperature, -1° to -3° C.
- 28th. }

June, 1915.

- 11th. } Night, minimum temperature, -7° C.
- to }
- 15th. }
- 16th. *Night*, minimum temperature, -5° C.
- 17th. Bright; maximum temperature in the sun, 28° C.
- 18th. *Night*, minimum temperature, -5.5° C.
- 19th. *Night*, minimum temperature, -6.5° C.
- 20th. *Night*, minimum temperature, -8° C.
- 21st. *Night*, minimum temperature, -5° C.
- 22nd. *Night*, minimum temperature, -6° C.
- 23rd. *Night*, minimum temperature, -6° C.
- 24th. Bright, dry; maximum temperature in the sun in the morning, 30° C.; in the afternoon, 40° C.; *night*, minimum temperature, -7° C.
- 25th. Bright; maximum temperature in the sun, 42° C.; maximum temperature of the room, 13° C.; *night*, minimum temperature, -8° C.
- 26th. Maximum temperature in the sun in the morning, 35° C.; in the afternoon, 46° C.; *night*, minimum temperature, -6° C.
- 27th. Maximum temperature in the sun in the morning, 30° C.; in the afternoon, 42° C.; maximum temperature of the room, 12° C.; *night*, minimum temperature, -8.5° C.
- 28th. As recorded on the 27th; *night*, minimum temperature, -7° C.
- 29th. As recorded on the 27th; *night*, minimum temperature, -7° C.
- 30th. In the morning dull; maximum temperature of the air, 20° C.; in the afternoon, bright; maximum temperature in the sun, 39° C.; *night*, minimum temperature, -5° C.

July, 1915.

- 1st. Bright; maximum temperature in the sun, 40° C.; *night*, minimum temperature, -6° C.
- 2nd. As recorded on the previous day; *night*, 9 p.m., temperature, 0° C.
- 3rd. Morning, 6 a.m., temperature, -10° C.; day, warm, genial; *night*, minimum temperature, -6° C.
- 4th. Warm, genial; *night*, minimum temperature, -6° C.
- 5th. As recorded on the previous day.
- 6th. Alternately bright and cloudy; fairly dry; *night*, minimum temperature, -3° C.
- 7th. Dull, rainy; maximum temperature of the air, 12° C.
- 8th. Dull, rainy; maximum temperature of the air, 10° C.; *night*, minimum temperature, 10° C.
- 9th. Dull, damp; maximum temperature of the air, 10° C.
- 10th. Dull, damp; maximum temperature of the air, 10° C.; *night*, clearing up; heavy dew; minimum temperature, -0.5° C.
- 11th. } Bright; damp in the morning; maximum temperature of the
- 14th. } room 15° – 18° C.; *nights*, damp; minimum temperature, 0° C.
- 15th. Dull; maximum temperature of the air, 14° C.
- 16th. Heavy rain; maximum temperature of the air, 16° C.; *night*, minimum temperature, 0.5° C.
- 17th. Alternately bright and cloudy; bright in the afternoon; maximum temperature of the air, 15° C.; *night*, cloudy; minimum temperature, 0.5° C.
- 18th. Dull and rainy; maximum temperature of the air, 11° C.; *night*, rain; minimum temperature, 0.5° C.
- 19th. Dull and rainy; *night*, rain; minimum temperature, 3.5° C.
- 20th. } Dull and raining.
- 21st. }
- 22nd. Bright in the morning, dull in the afternoon; maximum temperature in the sun, 36° C.
- 23rd. Bright; maximum temperature in the sun, 36° C.; *night*, minimum temperature, -8° C.
- 24th. } Bright; maximum temperature in the sun, 40° C.
- 25th. }
- 26th. Bright; *night*, 10 p.m., temperature, -2° C.
- 27th. 8 a.m., temperature, 4.5° C.; bright; *night*, minimum temperature, -3° C.
- 29th. Bright; *night*, minimum temperature, 1° C.
- 30th. } Bright; *night*, minimum temperature, -2° C.
- 31st. }

INFECTION.

Infection through the Mouth.

The direct introduction of mature larvae through the mouth of sheep is always followed by the presence of parasites in the stomach.

During the present work numerous lambs and sheep were infected with mature larvae of *Haemonchus contortus* either by giving the larvae

in water or with food, and invariably, in the examination of droppings or on post-mortem, an infection was found proportionate to the number of larvae administered.

Frequently mature living larvae were found in the droppings the day following the administration. A number of experiments were carried out by infecting lambs with eggs or larvae in the first and second stages, but the result was always negative.

Infection through the Skin.

A number of experiments were carried out, different methods being employed. Some of them were as follows:—

Experiment No. 1.—On the 7/10/14 a three-month-old lamb was selected, and the hair on the abdominal wall was cut very short over a patch the size of a hand. The patch was washed and dried, and was then kept moist for about 15 minutes with water containing a large number of mature larvae eight days old; the patch was then allowed to dry in the shade. After two hours the infected skin was well washed with a concentrated solution of lysol. During the two following days a slight reddening of the skin was observed, apparently produced by the lysol solution. From the 27/10/14 cultures of droppings were made from time to time, but with negative results. The lamb was killed on the 28/11/14, and no *Haemonchus contortus* were found in the digestive canal.

Experiment No. 2.—On the 8/10/14 a four-month-old lamb was selected, a patch was shaved on the abdomen, and a paste, made from black turf to which a great number of mature larvae had been added, was applied to the patch. The lamb was muzzled for six hours, and then treated with lysol as stated in Experiment No. 1. The lamb was killed on the 24/10/14, and no worms were found in the intestinal tract.

Experiment No. 3.—(a) On the 29/1/15 cultures were made from droppings of lamb No. 7161, four months old, which did not show the presence of larvae. The skin of the abdomen was shaved over an area 10 cm. square. On the skin so prepared a piece of calico soaked in water containing numerous larvae of *Haemonchus contortus*, mixed with larvae of *Strongyloides papillosus*, was applied. Over the calico a sheet of gutta-percha and a thick layer of cotton-wool were placed, and the whole was fixed to the shaved patch by a bandage. The lamb was isolated in a disinfected box. The following day the piece of calico was again soaked in water infected with larvae, and replaced on the lamb for twenty-four hours. The next day the shaved patch was washed with absolute alcohol and the lamb with an appropriate lysol solution. The lamb was then left in the box, and fed with food passed through an autoclave.

(b) On the 5th/12/15 lamb No. 7162, eight months old, was found to be free of larvae and was treated in the same way as No. 7161. The larvae were applied for three consecutive days.

Cultures from droppings were made every day, with the result that, nine days after infection, in both lambs larvae of *Strongyloides papillosus* were found. Larvae of the same species were very numerous in cultures made about 15 days after infection.

On the 3/3/15, 33 days after infection, lamb No. 7161 was killed, and in the stomach were found six *Haemonchus contortus*, which from their development appeared to be of a date anterior to the artificial infection.

Lamb No. 7162 died 21 days after infection. Five *Haemonchus contortus* were found in the stomach, apparently due to previous infection.

(c) On the 6/3/15 lamb No. 6415, eight months old, was infected by a similar method as mentioned above with larvae of *Strongyloides papillosus*. The larvae were taken from cultures of No. 7162. About 9 days later the culture from droppings showed larvae of *Strongyloides papillosus* rather frequent.

(d) On the 27/3/15 two other lambs were infected in the same way as mentioned above with larvae of *Haemonchus contortus*. The lambs were killed, one 20 days and the other 30 days after infection, and on post-mortem only a few old *Haemonchus contortus* were found in the abomasum, which appeared to be of a date anterior to the artificial infection.

Experiment No. 4.—Lamb No. 6421, four months old, had been treated with Cooper's dip and bluestone to clean it from wire-worms. On the 21/7/14 30 c.c. of water containing numerous larvae of *Haemonchus contortus* was injected in three different places subcutaneously. On the 29/7/14 water containing larvae of *Haemonchus contortus* was applied to the inguinal region, and allowed to evaporate, and the lamb was killed an hour later. In order to ascertain the result of the first infection, the three last stomachs, and the mucosa of the trachea and bronchi were examined, and no larvae were found. In the three places of injection a small caseous-purulent centre, surrounded by serous infiltration, was present in the subcutaneous tissue. In the centre numerous larvae were present with or without the outer skin. Some of them were alive.

In order to ascertain the result of the second infection, the infected skin was washed thoroughly and cut out. No larvae were found in the skin. No larvae were found in the corresponding subcutaneous tissue, or in the inguinal lymphatic glands.

Conclusions.—The above experiments show that it was not possible to infect lambs through the skin, although the method used proved to be effective for other species of larvae.

PARASITIC LIFE.

The subject of the following notes is the life of the larva after its entrance into the host. The period of time required for development, from the moment when the larva enters the stomach of the host until it reaches the adult stage, seems to be liable to variations, owing to influences not yet properly understood, but the average, however, can be estimated to be 15 days. Indeed it was not uncommon to find eggs in the faeces of the host even on the 15th day, thus indicating that the adult stage was reached earlier. On the other hand, however, it was noticed that notwithstanding the fact that some worms had reached the adult stage, oviposition was retarded for 10 days or more. The shorter period required by the parasite to reach sexual maturity was noted in a hot season, and the longer period in a cold season. From the morphological and biological point of view, the evolution of *Haemonchus contortus* within

the host can be divided into three stages, viz., the parasitic part of the third larval stage, or the first parasitic stage of the larva; the fourth larval stage, or the second parasitic stage of the larva; and the stage of the "adult" worm, or the third parasitic stage. During the parasitic development, three ecdyses are noted; the second ecdysis between the free and the parasitic part of the third stage, the third ecdysis at the beginning of the fourth stage, and the fourth ecdysis at the beginning of the fifth stage.

First Parasitic Stage (Parasitic Part of the Third Stage).

Biological notes (second ecdysis).—The mature, non-parasitic larva possesses a skin, which is very fragile, in the anterior portion of the body, and which can easily be removed in the shape of a hood. Under favourable conditions of moisture and heat as verified in the host, the larva shows a marked motility which assists a rapid ecdysis. Larvae introduced into sheep by means of water reach the abomasum, and undergo here the second ecdysis. The majority of the moulted larvae then take shelter in the stomach mucosa. Only a few are washed into the intestines. I was able to notice this fact in lambs, which, when slaughtered two days after drenching, showed a large number of larvae adherent to the mucosa, whereas only a small number were found in the faeces, some being dead. Taking into consideration the great number of larvae remaining, as compared with the small number washed down into the intestines, it is safe to conclude that the larvae possess a distinct biotactism for the mucosa of the fourth stomach. This peculiarity does not seem to hold good for the mucosa of the first three stomachs. In lambs infected with larvae reared on solid media, very few were found in the contents of the rumen between the 24th and 36th hours, and none at all were detected after a lapse of 48 hours, at which time the mucosa of the stomach was swarming with them. Further, no lesions were seen in the first three stomachs. Larvae introduced into sheep with solid food start at once the second ecdysis. The mouths of lambs, after feeding with grass on to which a culture of larvae had migrated, were rinsed out, and the water examined for larvae. Larvae with cast skins were found, thus indicating that the second ecdysis had taken place as soon as they entered the mouth of the host. As soon as the mature larvae have shed their skins they begin to feed. When the fourth stomach is reached they lodge between the minute epithelial processes of the mucosa, where they shelter without actually piercing the mucosa. This can be demonstrated in the following ways:—

- (1) When an animal is killed some time after it has been drenched with larvae, it can be seen, on manipulating the stomach, that the larvae drop off. When the mucosa is examined under the microscope the larvae still adhering can be seen moving between the minute epithelial processes of the mucosa.
- (2) Examination of the intestines of the larvae does not reveal the presence of any red corpuscles, nor of particles of the mucosa.
- (3) The buccal armature of the larvae is still cylindrical and unarmed.

Structure of the Larvae after the Second Ecdysis. Size.—It appears that in the parasitic part of the third stage the larvae do not increase in length

when compared with mature larvae of the free living stage. The following tables give some measurements to illustrate this :—

Measurements of free mature larvae, two months after hatching, during which time they were kept on the walls of a culture tube.

Length.	Thickness.	Tail.
μ	μ	μ
728	26.2	140
760	26.5	140
740	26.5	142
820	26.5	142
799	26.4	140
784	26.5	141

Measurements of larvae of the first parasitic stage, collected from the stomach of a lamb two days after infection.

Length.	Thickness.	Tail.
μ	μ	μ
655	22.4	75.6
666	21	76
760	21	78
756	22	70
784	22	75
792	22	78
840	22.4	75.8

A striking change that has taken place is the shortening of the tail the tip being 5–6 μ thick (Fig. 25). The shape of the worm is still cylindrical. The anterior end tapers slightly towards the mouth (Fig. 24). The skin is transversely striated. The lateral lines are 3 μ broad and slightly concave, ending anteriorly a few microns away from the tip of the head and posteriorly passing the anus.

Alimentary Canal.—The opening of the mouth is bounded by the anterior end of the skin (Fig. 24, cut. head). It is surrounded by six points, representing the cephalic papillae (Fig. 24, pap. dors.). The aperture of the mouth is triangular, and the lip is either open or closed. The lip measures 2–3 μ in thickness. The mouth cavity is still cylindrical and about 5–7 μ in length (Fig. 24, m. cav.). The walls are not rigid as in the free-living stage; when the larva is not feeding the walls are collapsed and the lumen is reduced to a line. In examining larvae in profile the head of the body has the shape of a pair of nippers, between which the mouth cavity lies longitudinally (Fig. 24, m. cav.). The space between the walls of the mouth and the branches of the nipper is occupied by a mass of granular tissue (Fig. 24, gr. tiss.). The substance of the two branches represents the primordium of the future mouth capsule (Fig. 24, m. cap.).

The Œsophagus.—The Œsophagus measures 180–195 μ in length by 8–10 μ in thickness in the posterior portion. A very slight constriction is still noted in the middle portion, but the general shape recalls a claviform body. The walls of the Œsophagus are transversely striated and the lumen

is linear (Fig. 24). The anterior end in the dorsal part shows a slight protuberance where the buccal lancet will appear in the fourth stage. (Fig. 24, buc. lan.). There is no change in the two circular valves behind the oesophagus.

The Intestines.—The intestine is still composed of 16 triangular cells filled with granulations and with nuclei slightly visible through the mass of the granules. The lumen is zig-zag shaped. The rectum is thin, situated obliquely to the main axis of the body and measures about 40μ in length (Fig. 25, rect.).

The Nervous System.—The nervous system does not show any remarkable changes other than an increase in number of the nuclei of the ganglia.

Excretory Apparatus.—Two large cells are now seen ventrally in the coelomic cavity, extending posteriorly to the oesophagus, and connected with the excretory pore by a long peduncle. These cells represent the cervical glands. On transverse section the excretory canal in the lateral bands is also detected. The excretory pore is situated about 140μ from the tip of the head.

The Genital Primordium.—The genital primordium is situated about 350μ from the tip of the tail, lying slightly obliquely to the longitudinal axis of the body and consisting of two fairly uniform masses of cells, each measuring 5μ in length.

The appearance of the larvae in the third stage is quite distinct from that of the larvae of the fourth stage, the tail now being straight and symmetrical (compare Figs. 25 and 28), and the mouth having a cylindrical shape (compare Figs. 24 and 27). They are easily recognizable, even under a low power, from the free-living mature larvae, chiefly by reason of the bluntness of the tip of the tail (compare Figs. 21 and 25–26).

Third Lethargus.—Whilst the larva is in the active parasitic period of the third stage it produces a slight mucous deposit on the mucosa of the fourth stomach, which covers and secures the larvae amongst the epithelial processes. It is in this condition of shelter that the larvae are found in the third lethargus. The commencement of the third lethargus cannot be so clearly defined as in the free stages. Nevertheless, I came to the conclusion that it is reached from the 30th to the 36th hour after the larva has gained access to the host, and lasts about 12 hours; in making post-mortem of recently infected lambs 24 hours after infection I could not yet find larvae in the lethargic stage. In other cases, within about 30–36 hours from infestation, about 50 per cent. of larvae were found in lethargus. In another instance at the 48th hour 70 per cent. were already in the fourth stage. The larvae in the third lethargus are more or less curved, completely immobile, and hard to waken; even under the strong reflected light from the microscope, they can remain immobile for ten minutes or more. In a piece of mucosa from an infected sheep kept for seven days at about 0.5° C. (the specimen was slightly putrefactive), the larvae of the third lethargus were still alive while active of the same stage were dead and partly decomposed. Some of the lethargic larvae of the specimen just referred to above kept in tap water between a slide

and cover glass at 20° – 25° C. were still alive after two days; they were subsequently killed for further observations. Larvae in the active parasitic period of the third stage are easily distinguished from larvae in the third lethargus by the following peculiarities: The former are much more lively and there are no changes in the structure. Larvae of the latter kind are motionless, slightly curved, and show more confused structural outlines, occasional differentiation of the old skin, loops of the œsophagus or intestinal lumen, appearance of the new mouth capsule, and an asymmetrical outline of the new tail.

Second Parasitic Stage (Fourth Larval Stage).

Completion of the Third Ecdysis.—As stated above, the third lethargus lasts for about twelve hours, after which the larvae get rid of the outer skin. In observing some larvae undergoing moulting, I noticed that a longitudinal rupture of the old skin occurred at the bulb of the œsophagus through which the larvae gradually protruded sideways, withdrawing the head from the anterior end of the skin, and in doing so invaginating it like a finger of a glove. It is not uncommon to see a larva carrying the invaginated piece of skin about whilst it wriggles out of the posterior end of the skin (Fig. 26, out. sk.). I am not quite certain whether this is the normal mechanism of the third ecdysis, although the structures which are present in the mouth at this stage would indicate that this is so. The cast skin frequently shows a long cuticular film, hinged internally to the mouth opening. This represents the inner wall of the mouth cavity and a small piece of the lining of the œsophageal lumen (Fig. 26, cut. oes.). Other larvae are seen free from the old skin, but show remnants of the buccal tube and its cellular surroundings still adherent to the new mouth capsule. This fact might be taken to indicate that it is somewhat difficult for the anterior parts of the skin to separate from the new mouth cavity.

Larvae in the third ecdysis, or just after completion, were found to be from 750 – 850μ in length by 22 – 25μ in thickness. It appears consequently that but little or no growth is noted in the third stage. The object of this period of life seems to be the formation of a mouth apparatus adapted to piercing the mucosa of the stomach and thus to enable the larva to resume its parasitic life as a blood sucker.

Structure of the Larvae in the Fourth Stage.—The growth of the larvae in this stage appears to be very rapid, as shortly after the third ecdysis many larvae are found to reach 950μ in length, and a few measure 1 mm.

External Appearance.—The only peculiarity worthy of note is that the tail is asymmetrical smooth at the tip and constantly curved dorsally. (Figs. 26–28.)

Digestive System.—The mouth now shows a provisional capsule which lasts until the appearance of the mouth proper. The cavity has the form of a truncated cone with the bottom of the mouth as the base, measuring 3.5μ in diameter, and the top measuring about 2μ formed by the mouth opening (Fig. 27, m. cav.). The internal wall of the mouth observed from the lateral side appears more refrangent, indicating a thickening of the cuticular layer.

The Œsophagus.—The Œsophagus measures 160–200 μ in length, about 13 μ in width anteriorly and 20–23 μ posteriorly, the general shape being claviform. The lumen of the Œsophagus is narrow and regular, the walls are thick and transversely striated. The intestinal valves form two rings with a combined thickness of about 3–4 μ . The intestine is 700–800 μ long, 20–25 μ thick at the anterior end, decreasing gradually until the posterior end where it is 5–8 μ thick. The intestinal cells still number sixteen, are triangular in shape with granular protoplasm frequently showing two nuclei. The lumen is still of a zig-zag shape, but slightly funnel-shaped anteriorly and uniform in diameter in the rest of its course.

The Nervous System.—The nervous system shows no changes.

The Genital Primordium.—The genital primordium is situated about 250–300 μ from the tip of the tail. It forms an oval mass of 18–25 μ in length, containing twelve nuclei and makes a marked dent into the intestines. No other changes worthy of note could be detected.

Biology of the Fourth Stage: Further Structural Changes.—The larva in the fourth stage attaches itself to the mucosa by means of the buccal aperture and produces a small haemorrhage; a coagulum is produced which surrounds the worm. The coagulum is mixed with mucus and food particles. This phenomenon is observed about three days after drenching with mature larvae. The coagula are frequent and measure about 1–2 mm. When placing a piece of mucosa under the microscope and dissecting the coagulum, the larvae can be found on the surface of the mucosa covered by the coagulum. They are coiled up and remain in this position for three or four days when the blood coagulum appears to have increased in size. Together with larvae of the fourth stage, numbers of larvae of the third stage are still found on the mucosa about the 3rd day, but very rare. The body of the normal larva has now appreciably grown. Moreover, it appears that there is a difference in size co-ordinating with some constant structural changes. In larvae of the fourth stage, two distinct positions of the genital primordium can be found, i.e. shorter larvae with a longer primordium and more distant from the anus (Fig. 29), or longer larvae with a shorter primordium situated near to the anus (Fig. 30). This probably indicates the first differentiation of sex, the former being the males and the latter the females. At this stage of development, which is usually noticed on the 4th day after infection, the majority of larvae exceed 1 mm. in length and a few may even reach 1½ mm., with a thickness of 27–5 μ . The mouth is well developed, the lumen of the intestine has a wavy appearance, the cells are well-defined and filled with granulations. Between the 4th and 6th days the coagulum increases in size, thus probably indicating further irritation by the larvae. They are now frequent in the coagulum itself. The tail is bent like a hook, and the larva is apparently anchored to the coagulum.

4th to 6th Day.—Between the 4th and 6th days the male can be recognized by the thick posterior end, the tail being short, conical, smooth, and slightly curved posteriorly (Fig. 31). No details of the bursa can yet be detected. The genital primordium is transformed into a tube situated on the ventral side of the coelomic cavity (Fig. 31, gn. pr.).

In the female the spindle shaped genital primordium produces at the level of its middle section a slight swelling in the ventral side of the body (Fig. 32). Its medial portion consists of cells grouped in pairs, and both distal portions consist of a file of single cells (Fig. 32, gn. pr.). The tail is longer than in the male, tapers slightly and is bent dorsally (Fig. 32).

6th Day.—On the 6th day a distinction between the two sexes can easily be recognized under a low power.

Size.—In the male the length is from 2.7 to 3 mm., with a thickness of from 55–60 μ ; the tail is 50–70 μ long. In the female the corresponding measurements are 3.7 to 4 mm. long, 70–73 μ thick, and a tail length of 132 μ .

The Cuticle.—The cuticle is well developed, measuring about 3.5 μ to 5 μ in thickness, but it is not yet possible to detect detachment from the body, and only in rare cases are there any signs of the commencing separation of the tail of the male. The distance between the transverse striations of the skin is about 2.5 μ . The buccal cavity has a globular appearance, and the walls appear thicker than previously. The œsophagus does not show anything of interest. The intestinal walls have a finely granular appearance, and border a wide lumen. In the male sexual organs, one can now follow the length of the testes for a distance of about 800 μ , but only reaching within about 100 μ of the rectal sphincter. The primordium of the pulvillus postanal, the spicules, and the gubernaculum appear among the other structures of the posterior end as cellular masses with distinct outlines. The thick posterior end of the body has a transverse dorso-ventral sulcus bordered laterally by two protuberances 80 μ wide laterally, containing the primordium of the lateral lobes of the bursa. The tail commences at the dorsal commissure of the sulcus, and has a wavy appearance; it is roughly triangular in shape, measuring 70 μ in length (Fig. 33). In the female, at the level of the ventral protuberance previously described, a line of demarcation is noticed separating the linguiform process from the wall of the body. In the sexual primordium a differentiation can be made between the vagina, uterus, and ovary.

7th Day.—By this time the female has reached a length of 4.5 to 5 mm., with a thickness of 92–95 μ . The male measures 3.5 to 4.1 mm. in length, and 80–85 μ in thickness. The principal changes are—

- (1) A more marked appearance of the canalis testicularis; throughout its length two distinct rows of cells are seen, without any apparent differentiation; the anterior end is blind, and the posterior end joins the rectal ligament (Fig. 33, gn. pr.).
- (2) Of the two spicules the posterior end is distinct, whilst the anterior end verges into a long transparent baglike body (Fig. 33, spic.).
- (3) The lateral lobes of the bursa are now clearly seen through the old skin, appearing like two fans, but the rays cannot yet be definitely recognized (Fig. 33, burs.). In the medial lobe the bifurcation of the ray can be recognized.
- (4) In the female the ovaries are about 320 μ long, ending in a blind sac.

- (5) The primordium of the uterus is already recognizable by the transverse disposition of the cells of the internal lining, and is about 200μ long (Fig. 34, ut.).
- (6) The ovijector is represented by a distinct group of large cells (Fig. 34, ovij.).
- (7) It is also possible to detect the site of the opening of the vulva (Fig. 34, vulv.).

The stomach of a sheep examined at this stage does not reveal the presence of any free worms. The coagula are still present and may reach 5 mm. in diameter.

9th Day : Aspect of the Stomach.—The coagula have not increased in size or number, but are rather more flat, contracted, and darker in colour, the absence of haemorrhage indicating that the parasite no longer irritates the mucosa. The larvae are as a general rule still within the coagulum, and three kinds of larvae can be distinguished, namely, those in the fourth lethargus (70 per cent.), fourth ecdysis (15 per cent.), and the adult stage (15 per cent.).

Fourth Lethargus : Aspect of the Worm.—The larvae are coiled up in the substance of the coagulum, either isolated from one another or in some cases attached longitudinally in twos or threes, and in the latter case it is difficult to separate them from one another without breaking. Movements of larvae in this stage are not shown, and even under the microscope the larvae do not move at all unless roughly handled. When they are undisturbed, many hours can pass without any movements being detected. This is in striking contrast to other individuals in the same stomach, which have not reached the lethargic stage, or which have passed it, when they are seen to move about actively, and react sharply to the light of the microscope. The larvae found in the above conditions are evidently in the fourth lethargus. In fact, on examining their structure they are found to be undergoing important changes, although the new outlines of the different organs are not yet definite.

With regard to the provisional mouth capsule, the fundamental structure is unchanged, but the cavity is more globular (Fig. 35, or. cav.). The thickening of the walls is quite conspicuous (Fig. 35, kit.), and the mouth opening is funnel shaped (Fig. 35, marg. or.).

Adult Worm (Fifth Stage).

Completion of the Fourth Ecdysis.—The mechanism of this process does not differ in any essential from that of the previous stages. During the process of casting the skin, the larvae are usually found attached by the hooked tail to their surroundings. It can also be seen that the ecdysis of the female occurs a day or two later than that of the male. Considering that on the 7th day only a few larvae are in lethargus, that on the 9th day some are in the fifth stage, and that by the 12th day all have reached the fifth stage, it can be concluded that larvae of *Haemonchus contortus* undergo their fourth ecdysis between the 9th and 11th days, and that this ecdysis is preceded by a period of lethargus lasting for about twenty-four hours.

Male : Size.—The length of the body is usually over 5 mm., but rarely reaches $5\frac{1}{2}$ mm. ; the thickness is about 90μ .

Skin.—The cuticle is about $1\cdot5\mu$ thick ; the transverse striations are again very fine, measuring about 1μ , and extend from the head to the base of the tail. Longitudinal furrows can be found crossing the transverse striations. Special attention to the description of the skin surface will be given when the structure of the full-grown worm is under consideration. The cervical papillae are now situated at about 714μ from the anterior end, still adhering by the free portion to the body lines, and measuring about $35\text{--}40\mu$ in length.

Digestive System.—The mouth cavity corresponds in every particular to the mouth of the full-grown worm, although it is not yet completely developed (Fig. 36). It will consequently be described in the full-grown worm. It is about 12μ broad, with a buccal lancet measuring 11μ in length.

The Œsophagus.—The Œsophagus is club-shaped, and has a length of 750μ , and a width of 96μ at the base, and 24μ at the top. In the whole specimen the lumen appears linear, but rather irregularly shaped in the last part. The intestine is about 96μ wide in the first portion, and about 65μ wide in the posterior part. The chyle intestine walls are uniformly finely granular. The lumen appears to be irregularly shaped, owing to the pressure of the neighbouring organs.

In the male the rectum is about 15μ long, and is connected with the chyle intestine by a strong ligament slanting backwards on to the walls of the cloaca. The cloaca is about $40\text{--}50\mu$ long, being wider at the base and narrower at the apex (Fig. 39, clo.). The ventral walls are supported by the substance of the ventral bands. At the bottom of the cloaca the rectum, the spicular canal, and the ejaculatory duct are distinct. The genital cone is 50μ long by 37μ broad, and is covered by a thick cuticular layer (Fig. 39, ge. co.). The spicules are 320μ long, 16μ wide at the base and 4μ at the tip, with a knob-shaped head. The tip of the spicule does not project outside the cloaca, but is protected by a well-marked, hook-shaped protuberance of the dorsal wall of the cloaca (Fig. 39, spic.). The testes, the seminal vesicle, and the cement gland together form a long tube, commencing at about 1·5 mm. in front of the anus, about 32μ broad, lying ventrally to the chyle intestine and running straight to the cloaca. From the external appearance it is possible to distinguish an anterior part about 500μ long, containing two rows of large cells, which are probably the testes. For the following 50μ the tube is slightly enlarged, with uniform contents, representing the seminal vesicle ; the remainder represents the cement glands, and shows two rows of cells arranged like the barbules of a feather (Fig. 39, gl. cem.). The bursa is structurally well-developed, with the rays quite distinct, but more bunched together than in the later stages of the worm. The lateral lobes are 130μ long by 260μ broad, and the dorsal one is 88μ long by 32μ broad (Fig. 39, burs. l. lob., burs. d. lob.).

The Female : Size.—Just after the fourth ecdysis, the female measures between 6·5 to 7·9 mm. in length, although this maximum figure is very rarely reached. The thickness varies from 90μ to 130μ .

The skin and digestive canal do not show any marked structural differences from those of the male.

The Genital Organs.—In a female worm 7·9 mm. in length, the vulva is situated about 1·5 mm. from the tip of the tail. Externally the vulva opening is protected by a linguiform process, which measures 92μ in length and is 34μ broad at the base, with the tip removed about 60μ from the body walls. The vagina is a funnel shaped tube about 70μ long, directed forward with an opening into the unpaired part of the genital tube. The lining of its walls is folded. The opening of the vulva is surrounded by a slight protuberance of the cuticular mantle of the body, which will be described under the full-grown worm. The genital tube of the female consists of two portions. One portion is situated anteriorly to the vulva, and the other one is mainly situated posteriorly. Each portion consists of a uterus and of an ovary. Consequently, in the following pages, they will be spoken of as the *anterior genital* and *posterior genital tubes*. The paired called the *unpaired genital tube or canal*. The anterior genital tube is at this stage frequently distended, and runs ventrally between the chyle intestine and the wall of the body. The end of this anterior tube is situated about 1·3 mm. from the vulva, and the following divisions may be noted in it, from the anterior end backwards.

Ovary.—The anterior portion of the ovarian tube is about 170μ long and 24μ thick; it contains cells regularly arranged, resembling the cones of a fir tree. The succeeding tract of the ovary is about 400μ long. The lumen is large and uniform in diameter; the inner lining is composed of six longitudinal rows of cells, appearing trapezoidal in lateral projection.

The Uterus.—The uterus follows the ovarian tube, and is composed of a first portion, which is the uterus proper, and a second portion or muscular appendage called the ovijector. The uterus measures 220μ in length, the first half is 22μ thick and is homogeneous; the second half is funnel-shaped, with the base towards the ovijector, where it measures 430μ in thickness. The walls of the uterus show a peculiar arrangement of the epithelial cells of the internal lining (Fig. 37, ut.).

Ovijector.—Two portions structurally distinct can be recognized in the ovijector, viz., Pars haustrix and Pars ejectrix.

Pars haustrix.—This is the portion following the uterus and is roughly bottle shaped, with the base anteriorly situated. It measures 132μ in length, with a diameter of 44μ at the base, and $20\text{--}22\mu$ at the neck. The internal lining and the external muscular layer are not yet completely distinct. Of the internal lining four large nuclei are seen in the anterior portion.

Pars ejectrix.—The second portion of the ovijector is about 198μ long. The anterior diameter is 64μ and the posterior one is 36μ . The muscular walls are stronger than in the pars haustrix, particularly anteriorly where they are twice as thick as in the rest of the organ. The internal walls show large irregular folds, and at the posterior end the folds become longitudinal, forming a kind of plug, which protrudes into the unpaired genital tube.

Unpaired genital canal.—This portion is already distinct from the two uteri. The structural composition is not yet complete in all details, but a cuticular wall, an internal folded lining and a granular external layer are formed.

Posterior genital tube.—This differs but slightly from the anterior one. The ovary commences at a distance of about 256μ from the anus, proceeds ventrally and then diverges to the lateral side, where after making a few coils, it returns to the ventral side. When stretched out the ovary measures about 1 mm. Of this distance the germinative portion occupies 160μ , a similar length to that seen in the anterior one. The remaining 940μ is of the same structure as the anterior corresponding part. The uterus and the ovjector are of the same size and of a similar structure to the anterior one.

Nervous System.—Apart from the growth of the body resulting in increased numbers of nuclei, no other details worth mentioning were detected.

The Excretory System.—No further changes have to be recorded.

12th Day.—In examining the stomach of a sheep twelve days after infection, numerous coagula are present, but they are now more contracted and of a black colour adhering to the mucosa, whilst petechiae of recent origin are usually found under the coagula. These petechiae are caused by the piercing of the mucosa by the new mouth apparatus of the larvae, and differ from the one described previously, because they are punctures without any escape of blood. All worms are now found in the fifth stage, and are either in the coagulum, or between the coagulum and the mucosa. It is rare to find worms outside the coagula.

Male.—The length varies from 7 to 8 mm. by 100μ to 130μ in thickness. With the exception of a proportionate increase in size, the organs of the body do not show any new peculiarities. The genital canal is usually distended, measuring about 3.2 mm. in length by 30μ in width. The spicules are about 356μ in length. The genital cone is well developed, protruding for about 60μ from the floor of the bursa. The maximum length of the lateral lobes is about 165μ .

Female : Size.—The length varies from 9 to 10 mm. by 100μ to 150μ , with a tail length of about 250μ . The tip of the anterior genital tube is situated about 2.244 mm. in front of the vulva; the tip of the posterior tube is 1.192 mm. behind the vulva, but the tube itself when stretched is 2.8 mm. long. There are no points of interest in the development of the other organs.

15th Day.—The stomach of a lamb infected fifteen days previously still shows in some cases dark red coagula adhering to the mucosa, whilst in other cases the coagula consist of a residue of yellowish fibrine and ingesta mixed together. In all cases the worms are found in between the coagula, or distributed on the surface of the mucosa itself. Some worms are now found in copulation.

Male.—The male worm measures from 9 to 10 mm. in length with a thickness of 118μ to 140μ . The genital tube is 3.5 mm. long. The vesicula

seminalis mentioned above as a simple dilatation between the testes and the cement gland is now well defined at both ends, and frequently contains a mass of spermatozoa. The spicules are 396μ long and the gubernaculum is distinct.

Female.—The female varies from 12 to 14 mm. in length, with a thickness of 150μ , the tail length is about 720μ , and the vulva is situated at a distance of 3.1 mm. from the tip of the tail. A fact which immediately strikes the observer is that the two ovarian tubes have grown considerably in length. This is especially the case with the posterior one, which has turned round and now proceeds anteriorly to the vulva, nearly reaching the tip of the anterior tube at a distance of 5.180 mm. from the vulva. The several sections of the genital tube can now be distinguished definitely, and will be described under the heading of the full-grown worm. The second point of importance is that eggs have now passed through the genital tube, some of them having reached the ovijector. From these notes it is evident that the worms have commenced to lay eggs; in fact, it is sometimes possible to find eggs in the stomach contents.

18th Day.—When examining the stomach of a sheep 18 days after infection, all the coagula and fibrine deposits have practically disappeared. In a heavy infestation the mucosa is swarming with adult worms. The petechiae are numerous and small, but without any fresh blood coagulum. Numerous worms are in copulation, and numbers of females have laid eggs.

Male.—The male measures from 12 to 13 mm. in length. The thickness at the base of the oesophagus is 132μ , and at the base of the spicules is 198μ , gradually decreasing in diameter from the posterior to the anterior end. The genital tube is about 9.6 mm. long, ending anteriorly at a distance of about 4.15 mm. from the head.

Female.—The female is about 17 mm. in length, with a thickness of 150μ at the base of the oesophagus, and 257μ in front of the vulva. Behind the vulva the body is about 145μ thick, increasing slightly until the maximum of 231μ is reached, and then decreasing until the minimum is reached at the tip of the tail. The vulva is situated at about 2.5 mm. and the anus at about 390μ from the tip of the tail. The anterior portion of the rectum is markedly enlarged, showing an internal lining with transversal folds. The striking change of the genital organs is the increased size of the ovary and its spiral shaped disposition around the chyle intestine. The anterior ovarian tube runs for a distance of 7.9 mm., and ends at a point 4.2 mm. from the head. The posterior ovary ends at 1.6 mm. behind the anterior one and extends for a distance of 8.1 mm. The posterior ovarian tube, considered from the point of connection with the posterior uterus, turns forward and runs parallel to the left side of the chyle intestine until it meets the pars haustrix of the anterior uterus; from this point it turns dorsally and then laterally to the anterior part of the uterus, and on the ventral side meets the commencement of the anterior ovarian tube. From here the two ovarian tubes run round the intestines, making about nine spirals. The uterus is distended by eggs,

which are also present in the unpaired part up to the vagina. In older parasites no other essential structural changes were observed, except normal development.

ANATOMY OF THE ADULT WORM.

Method of preparing Specimens for Examination.

Living specimens were used to observe the functions of the organs. Fresh specimens were used in studying some details of the skin, nervous and excretory systems, which were changed by the fixing process. Specimens cleared in glycerine, according to the method of Looss, proved to be more satisfactory for the study of the internal structure. Transverse sections by the paraffin method were attempted, chiefly for the study of the mouth. The process was found to be delicate, and the result was not always satisfactory. Transverse sections of specimens cleared in glycerine proved to be simple and useful. The method used first of all was as follows:—The portion of the worm to be examined was separated from the body and fixed in vertical position on a thin cube of animal tissue. This operation can be easily performed under the binocular microscope; the preparation was then placed on the freezing microtome. A few drops of water were placed on the preparation during the process of freezing, until the portion of worm was enclosed in ice. In later investigations the use of animal tissue was discontinued and the portion of worm was fixed vertically in a drop of water which was freezing on the microtome. The specimen was then enclosed in ice in the above way. When cutting the frozen mass a drop of water can be seen to appear on the blade of the razor. This drop of water was then taken up with a pipette and poured on a drop of glycerine on to a glass slide. A cover glass was placed on the glycerine and the preparation was examined.

If the above sections are embedded in gelatine and sealed with asphaltum they can be preserved for years. In this way a series of sections can be prepared. The sections must be about 15μ thick. A number of sections is always broken, but many of them are quite suitable for examination; sections of the anterior portions of the body along the oesophagus and the portion where the internal organs are pressed together proved more suitable than the posterior. For a quick examination fresh specimens can also be used for sections with the freezing microtome.

Size.—Variations in size of the specimens, due to the method of preservation and age of the worm must be expected. The following experiments with different methods of preservation were undertaken with a view to obtaining accurate figures:—

Wire-worms were collected in the beginning of spring, at a time when parasites were supposed to have been present in the stomach since the previous summer. The worms were collected from the stomach immediately after the sheep was killed, were measured and each pair of male and female worms was kept in a watch glass with water at a temperature of 24° to 25° C. They were measured again ten minutes later when they were found motionless, and to all appearances dead. Measurements were again taken forty hours later, when post-mortem changes were evident.

The following table indicates the various measurements recorded :—

EXPERIMENT No.	SIZE.						REMARKS.
	WHEN COLLECTED.		AFTER DEATH.		48 HOURS AFTER COLLECTION.		
	Female.	Male.	Female.	Male.	Female.	Male.	
	mm.	mm.	mm.	mm.	mm.	mm.	After 48 hours the females were swollen and the internal organs dis-integrated. The males were still in a fair state of preservation.
1	25	17	30	21	Broken	19	
2	25	15	31	18	23	17	
3	26	16	30	19	24	17·5	
4	25	18	25	21	Broken	19·5	
			(Broken)				
5	25	17	29	20	26	18	
			(Broken)				
6	29	16	30	19	27 (Broken)	17·5	

In the above table the following observations can be noted :—

- (1) The maximum length of *Haemonchus contortus* is recorded within a few hours after collection at a time when the worms appear motionless or dead, owing to the collapse of the body tissues.
- (2) When post-mortem changes have advanced the worms are again shorter, owing to swelling of the body.

The apparent discrepancy in the length of the male worm 48 hours after collection, compared with that shown immediately after collection, must be explained by the fact that males remain in a state of good condition for a longer period than females. Worms left in the dead body of the host are seen to die very quickly, especially in cases of toxæmic diseases; consequently their size is affected very soon after death of the host. The variations in the size of the worms under such conditions are more marked during the summer months, and especially when the carcass of the host is allowed to lie in a warm place. In sheep that died during the night, on which post-mortem examinations were made about ten to twelve hours later, the worms were generally found to be dead. They all had a collapsed appearance, and in full-grown specimens the minimum length of the female was 27 mm. and the maximum 33 mm. In sheep that died on the veld, and had been exposed to the sun, I frequently found the female worms with a length of 31 mm., although more often the specimens even had a maximum length of 33·5 mm., whilst the males measured 19 to 20 mm.

The following table gives certain measurements concerning the size of *Haemonchus contortus* when fixed and preserved :—

No. OF EXPERIMENT.	SIZE.					
	WHEN COLLECTED AND STILL ALIVE.		24 HOURS AFTER FIXING.		A MONTH AFTER CLEAR- ING BY GLYCERINE.	
	Female.	Male.	Female.	Male.	Female.	Male.
	mm.	mm.	mm.	mm.	mm.	mm.
1	25	16	25	17	26	17
2	27	16	27	17	27	18
3	26	15	26	16	27	16
4	27	17	27	17	29	18
5	28	15	29	16	29	16
6	27	16	28	16	28	17

The worms were all collected from the same sheep as mentioned in Table No. 1, and immediately after collection they were fixed in 70 per cent. alcohol, subsequently being cleared with glycerine according to the method of Looss. The following conclusions, which can be drawn from the above table, are also supported by numerous other observations made on fixed and preserved worms:—

- (1) When a living worm is placed on a slide for the purpose of being measured, the longitudinal contraction of the body is conspicuous, and can be explained by reflex action against the unfavourable ambient.
- (2) Killing the worms in 70 per cent. hot alcohol fixes the size practically at normal, as it prevents the post-mortem muscular relaxation.
- (3) The size shown by worms embedded in glycerine is probably the most correct, and it appears that glycerine is the best preservative for correct measurements of small worms.

The second factor to be taken into consideration for establishing the average size of *Haemonchus contortus* is the age of the parasite. The average size of the young adult worm is not the average size of the fully-grown worm. There is a difference in the two sizes, and the former cannot be taken as the size of normal specimens. *Haemonchus contortus* reach the adult stage after the completion of the fourth ecdysis, when the average length is 5 mm. in the male and 7 mm. in the female. These figures correspond to the initial stage of development in the adult worms, and represent practically one-third of the length of the fully-grown worm. The average sizes given later are taken from adult specimens in which development was complete. The difference between young and fully-grown *Haemonchus contortus* is easily recognizable under low magnifications by the more pronounced pinkish colour of the young worm, by the marked pigment deposit on the intestinal walls of the fully-grown worm, and by the difference in development of the genital organs. By taking the above factors into consideration, the date of infection of a host can be ascertained with a fair degree of certainty. The following notes are taken from one particular case, and illustrate the points I have referred to. In a sheep that died after a short death struggle, during the first part of the summer, numerous wire-worms were found in the fourth stomach in good condition. The worms were then separated into two lots, according to their external appearances. In the first lot the females measured 15 to 18 mm. in length, the males from 12 to 14 mm. Both sexes were pink in colour, and the ovarian tubes showed only a few coils round the intestine, thus indicating an incomplete development in length. In the second lot about 15 other specimens were collected. These were chiefly females, 27 to 28 mm. in length, with a brownish colour, a large store of pigment granules, and with the genital tubes conspicuous by their number of coils and thickness. The conclusion was that the first batch of worms had obtained access to the host in the spring just ended, and that the second batch were of a much earlier infection, which probably occurred before the previous winter.

The material I examined during the three years in which these observations were recorded, came exclusively from South Africa, and the majority of the specimens were collected by me personally from animals at

the Onderstepoort Laboratory. In forty cases the wire-worms were measured as soon as collected, being still in good condition, and they were measured again when preserved in glycerine. The average size of full-grown males was in this way established to be 15 to 18 mm., with a thickness of 164μ at the base of the oesophagus and 270μ near the base of the bursa. The average length of the full-grown female was established as 25 to 29 mm., the thickness at 171μ to 178μ at the base of the oesophagus, and 375μ to 382μ near the base of the linguiform process. The measurements of the thickness were made from worms either with or without a cover glass placed over them, whilst floating in the media. A large number of other cases were recorded in which the wire-worms were collected when dead or relaxed, but in a good state of preservation. From these specimens the length of the female was found to be 29 to 33 mm., and in many cases even 35 mm., but in the case of the male the increase was not so marked, the average length being 17 to 21 mm. Presumably worms in the above condition are frequently collected for identification, but such material would not be suitable for determining the size of the species. Assuming that the size of *Haemonchus contortus* is the same in the different continents, then the length of 10 mm. in the male, and 18 mm. in the female, reported by Stiles and Perroncito, and 13 mm. by Lewis in the male, probably apply to worms collected in the early days following infection. Females measuring 30 mm. in length, as reported by Railliet and Stiles, were never found by me under the above conditions, but worms of this length were frequently noted in specimens that had deteriorated.

Form of the Body.—The body of the male decreases in diameter slightly from the base of the bursa to the anterior end (Fig. 40). On cross section the periphery is not completely circular, but is slightly compressed dorso-ventrally (Fig. 44). In Figs. 46 and 47 the periphery seems to be deformed by the tension of the muscles along the lateral bands. In the female the body decreases in size from the base of the linguiform process to the head. Posteriorly the diameter of the body is largest midway between the vulva and the anus, and decreases in thickness on either side. From the anus, the tail is conical in shape, with the tip fairly pointed (Fig. 41). In transverse section the body is usually slightly compressed dorsoventrally, as is noted in the male (Figs. 51-53).

General Appearance of the Worm.—The main part of the body of young living specimens has a pale, transparent appearance. In the female there are two spirals within the body, one whitish and the other pinkish in colour; when the worms become older, the colour of the latter spiral changes to a slight brownish hue, and becomes more distinct. At the time of death the worms have a whitish or greenish appearance, and are more opaque. Specimens preserved in alcohol are quite white and opaque, whilst those preserved in glycerine are yellowish and markedly transparent.

Body Walls: Cuticle.—The cuticular layer varies from 11μ to 16μ in thickness. In the male, at the level of the cervical papillae, the cuticle is 13μ thick dorso-ventrally and 28μ laterally. In the female the thickness of the cuticle reaches 70μ in the dorsal part of the linguiform process of the vulva (Fig. 52) and 7μ at the tip of the tail. The external surface of the

cuticle is transversely striated, each striation being between 1.5μ and 3μ broad, slightly narrower and less marked towards the two ends of the body, but remaining visible until the level of the buccal capsule (Fig. 42). A number of longitudinal furrows divide the cuticular surface into bands, measuring 25μ to 33μ broad, and slightly convex medially. These furrows are not constant in breadth, but vary according to the movements of the worm. In transverse sections examined either in fresh worms or in those cleared in glycerine, the appearance of the cuticular surface is seen to be quite different to the above description. Figures 43 to 47 were drawn without correction by the Abbe apparatus. The difference is due to the fact that, in the unavoidable deformation of the cuticle during the fixing and clearing operations, the skin became wrinkled, taking the peculiar disposition seen in the figures referred to above, where the cross section of the longitudinal furrow is represented by the external angles, and the width of each band is represented by the concave tracts. This deformation is so constant in section that it could easily be mistaken for the natural configuration of the skin. In Figs. 51, 53, and 56, the external appearance of the cuticle was corrected according to the appearance seen in the live worm.

Chitinous Rod.—The chitinous rods mentioned by Leukart and Schultness and described by Looss in the *Ankylostoma* are conspicuous in *Haemonchus contortus*. In whole specimens they appear as narrow more transparent strips running in the middle line of the lateral bands from the pre-cerebral portion of the œsophagus to the base of the bursa in the male and posteriorly to the anus in the female. In cross section the chitinous rods appear as small discs about 8μ in diameter and situated between the cuticle and the base of the lateral bands (Figs. 43–46, kit.).

Subcuticle.—The subcuticle is represented by a thin granular layer, lying between the cuticle and the muscular cells (Fig. 46, cut. int.), protruding between the muscular quadrants into the coelomic cavity, where it forms the longitudinal bands. In this way are formed one dorsal, two lateral, and one ventral bands.

Dorsal and Ventral Bands.—The dorsal band commences in the dorsal tip of the mouth. At the level of the connection of the œsophagus with the mouth the dorsal band reaches the wall of the œsophagus (Fig. 42, b. dors), where it diverges laterally and forms a thick granular layer around the dorsal part of the œsophagus (Fig. 43, b. dors.). The ventral band commences at the commissure of the two subventral lips of the mouth. At the level of the connection of the œsophagus with the mouth it is broader than the dorsal band (8μ), reaching the ventral walls of the œsophagus, to which it seems to act as a kind of support (Fig. 43, b. vent.). In its substance, nuclei are present, which are sometimes quite distinct. At the level of the nerve ring the two bands are seen in transverse sections to be narrower at their base (Fig. 44), the dorsal one touching the nerve ring from where it diverges on both sides. Between the two bridges of the dorsal band, the dorsal cephalic ganglion is seen (Fig. 44, gl. dors.). The ventral band supports the nerve ring. On both sides are two nucleated cells, apparently representing the "Ventral adventitious cells of the nerve

ring" (Fig. 44, c. adv. ven.). At the level of the cervical papillae the dorsal band is still connected with the œsophagus, and the ventral one appears connected with the vesicle of the excretory system (Fig. 45, b. ventr.). At the level of the posterior end of the œsophagus the median bands are still rather wide, but are compressed dorso-ventrally by the œsophageal bulb. Posteriorly to the œsophagus their breadth is reduced to 3μ or 4μ , apparently due to the pressure of the muscular cells of the body walls (Fig. 46). Within the body cavity they reach the level of the muscular cells. Posteriorly no further peculiarities can be detected in the medial bands, except in the ventral one, which in the female just anteriorly to the vulva gives origin to the granular internal portion of the linguiform process (Fig. 52). In the posterior part of the body the median bands very much resemble the disposition of similar bands in *Ankylostoma duodenale* described by Looss. In the female, just posteriorly to the vulval opening, the ventral band again appears considerably increased in breadth and thickness, reaching the opening of the anus. In the same region a granular oval-shaped mass, showing nuclei, is found protruding dorsally from the rectum into the coelomic cavity, forming the so-called pulvillus post analis, which posteriorly decreases in size until it reaches the tip of the tail (Fig. 58, pulv. p.a.). In the male the ventral band is again increased in thickness at the posterior end of the body. At the level of the curvature of the ventral wall of the body into the floor of the bursa the ventral band encircles the cloaca and joins dorsally, forming the Pulvillus post analis. The dorsal bands gradually decrease in thickness towards the posterior part of the body without any peculiarity.

Lateral Bands.—The lateral bands take their origin in the sublateral lips of the mouth capsule. Posteriorly to the mouth they appear in cross section as broad as the dorsal band. They protrude into the body cavity, dividing into a dorsal and ventral bridge. The dorsal bridge supports the cephalic gland and is connected with the bridge of the dorsal band (Fig. 43, gl. ceph.). In the sections under observation it appeared that the bridges of the four longitudinal bands were fused together at the same level, forming the cephalo-œsophageal ligament as observed in other nematodes (Fig. 43). The ventral bridge of the lateral bands is thicker and contains the anterior portion of the excretory canal. At the level of the nerve ring the lateral bands are broader than the two medial ones. The bifurcation surrounding the cephalic glands is well marked. The dorsal bridge is connected with adventitious lateral cells that have been described in other nematodes (Fig. 44, c. adv. lat.). Slightly behind the nerve ring, the dorsal bridge of the lateral bands is very much compressed by the cephalic glands (Fig. 45). The ventral bridge supports the termination of the excretory apparatus (Fig. 45). At about the same level the lateral bands give origin to the granular cone of the cervical papillae (Fig. 45). At the level of the posterior end of the œsophagus the lateral bands are reduced to a thin granular layer by the compression of the cephalic glands. Proceeding backwards, in the male the lateral bands are reduced in breadth (Fig. 46). At the level of the spicules they are again broader and appear in cross section as a granular pedunculated mass (Fig. 47, b. lat.). At the base of the bursa the same bands make a dorsal and a

ventral diversion and contribute to the formation of the pulp of the bursa. In the female the lateral bands as seen between the posterior end of the œsophagus and the anus are broader than in the male (Figs. 51, 53, and 56). Posteriorly to the rectum they form the main substance of the tail on both sides as far as the tip (Fig. 59).

Sub-Lateral Bands.—The sub-lateral bands are inconspicuous in the preœrebral portion. At the level of the cervical papillae the four sub-lateral bands are easily detected (Fig. 45, sub. 1.), but are small in size. From the posterior end of the œsophagus they are constantly seen in cross section between the large and small muscular cells running along the lateral bands (Figs. 46, 51, 53). On cross section in the male at the level of the spicules the sub-lateral bands are much increased in size (Fig. 47, b. sub.). Posteriorly they take part in the formation of the pulp of the bursa.

Musculature.—The somatic musculature of *Haemonchus contortus* is of polymyarian type, the usual number of cells in each quadrant being 8 to 9 (Figs. 46–47). The muscle cell is of coelomyarian structure, except the cell between the lateral and sub-lateral bands, which appears distinct from the other cells of the same quadrant, showing some platymyarian characters (Figs. 47–51). The muscle cell of the first type has an average length of 1.5 mm. It is narrow, gradually decreasing in breadth towards the two ends (Fig. 41A, coel. 1, coel. 2), and protrudes conspicuously into the coelomic cavity (Figs. 46–47, coel.). The fibrillar striation is perpendicular where the cell adheres to the walls of the body and transverse on the two sides. The little protoplasm present is on the internal margin of the cell (Fig. 46–47). Frequent sarcoplasmic processes are present between two or more cells. The cell of the second type is broad at the base, occupying in certain parts of the body all the space between the lateral and sub-lateral bands (Fig. 41A, plat. 1, plat. 2). The fibrillar striation is vertical to the base. The granular part is abundant on the centre of the cell, showing a large nucleus (Figs. 47–51). The length of the cell is sometimes 4 mm. In transverse section frequently two muscular cells are seen in pairs between the lateral and sub-lateral bands, but are unequal in size. They represent the ends of two consecutive cells as represented in Fig. 41A, plat. 1, plat. 2. The somatic muscular cells adhere to the sub-cuticular layer of the body walls and are disposed longitudinally, pressing against each other. Throughout the length of the worm, the muscular coat can be divided into a series of segments or rings with saw-shaped edges. Each ring is formed by muscular cells of similar lengths, ending at the same level. As a result of the protrusion of the longitudinal bands, the muscular coat is divided into quadrants. The transverse section of a muscular ring is represented in Fig. 46. A longitudinal projection of one of the muscular quadrants is given in Fig. 41A. Each ring fits in with the adjoining one in such a way that the pointed ends of the muscular cells lie between the ends of corresponding cells of the adjoining ring. Fig. 41A shows the manner in which the cells of a quadrant fix into the ends of the corresponding cells. In Fig. 47 the two dorsal quadrants show the transverse section of the two muscular rings, in which the posterior end of the anterior cells can just be recognized. At the base of the lips in the male, one muscular cell is seen in each quadrant and

on cross section appears triangular. A few microns behind a second cell is found in each quadrant between the previous one and the lateral band. The eight resulting cells were recognized to correspond to the cephalo oesophageal cells already described by Looss in the *Ankylostoma duodenale*. In fact, their shape shows a tendency to be cylindrical, as in cross section the fibrillar layer completely surrounds the sarcoplasm (Fig. 43, m. ceph. oes. a. and p). The above cells end on the oesophageal walls below the nerve ring. Very soon after the anterior insertion of the cephalo oesophageal cells some more cells are found in each quadrant, and at the posterior end of the oesophagus seven of them can be counted. More posteriorly there are seven to eight in each quadrant. In cross section the cells are frequently more numerous in a quadrant, the cells of two consecutive rings being cut (Figs. 47-51). The number of the rings of muscular cells was counted, and their lengths measured. In a male of 17 mm. in length the arrangement was as follows:—1st cell appearing after the anterior origin of the cephalo oesophageal cells 499μ in length. 2nd cell appearing after the anterior origin of the cephalo oesophageal cells 520μ in length. The 3rd cell appearing after the anterior origin of the cephalo oesophageal cells 650μ in length. The next cells commence at the level of the posterior end of the oesophagus. The following figures give the length of the nineteen rings counted between the posterior end of the oesophagus and the base of the bursa:—1st, 715μ ; 2nd, 928μ ; 3rd, 1.071 mm.; 4th, 1.213 mm.; 5th, 1.428 mm.; 6th, 1.570 mm.; 7th, 1.785 mm.; 8th, 1.785 mm.; 9th, 1.785 mm.; 10th, 1.785 mm.; 11th, 1.785 mm.; 12th, 1.785 mm.; 13th, 1.785 mm.; 14th, 1.785 mm.; 15th, 1.428 mm.; 16th, 1.428 mm.; 17th, 1.071 mm.; 18th, 714μ ; and 19th, 714μ .

Counting from the posterior end of the oesophagus to the tail, ten platymyarian cells were found between a lateral and a sub-lateral band. The maximum length was 4 mm. in the middle of the body, two consecutive cells lying along side each other for a distance of 1.7 mm. The two posterior ones were one-third of the length of the anterior ones and indistinguishable from the bursal muscles. Figure 47 shows in cross section the change of the ventral muscular quadrants at the level of the genital organs. In a female 25 mm. long, the muscular cells along the oesophageal portion of the body were similar to those of the male. From the posterior end of the oesophagus until at the vulva, 25 consecutive muscular rings were counted, the longer cells measuring 2.2 mm. From the vulva backwards five to six rings of cells were counted, the cells being short and narrow. Figure 56 shows in cross section that the muscles of the two ventral quadrants, situated at the level of the vulva have nearly disappeared. It was further noted in cross sections of the female that posteriorly to the vulva the two ventral quadrants are again complete, although the muscular cells are smaller in size. Behind the posterior uterus the cells in each ventral quadrant number four, and further back only two, the rest of the quadrant being occupied by the lateral and ventral bands. At the level of the rectum only one muscular cell appears dorsally, and two cells ventrally, all of which are reduced to the fibrillar part. The above somatic muscles disappear in the tail.

Digestive System.

Mouth : Mouth Aperture.—The oral opening is triangular, each side of the triangle representing a lip. One lip is dorsally situated, and the other two are sub-ventral. The three lips are separated by an incision which extends as far as the cephalic papillae, of which each lip supports two. Between each pair of papillae is a groove, running along the medial axis of the lip to the edge. The edge of each lip is formed by the medial axis of the lip until the edge. The edge of each lip is formed by external cuticle of the head, which projects with a sharp fold towards the mouth cavity. In Fig. 42 the above fold is seen in the dorsal lip in lateral projection, and in the left sub-ventral lip in frontal projection. The incision separating the lips enables the oral opening to expand. In examining a number of specimens under high magnification, the lips are found in different degrees of inclination toward the mouth cavity. In Fig. 42 the lips are slightly distended.

Mouth Capsule.—The mouth capsule is formed by a thick layer of chitin. It takes origin anteriorly just behind the edge of the lips, where a slight sulcus (not reproduced in Fig. 42) is formed. From here the mouth capsule reaches the anterior tip of the œsophagus. The mouth cavity has the form of a truncated triangular pyramid, with a dorsal and two sub-ventral sides. The base of the pyramid corresponds to the bottom of the mouth. The space between the sub-ventral walls of the mouth capsule and the cuticle of the head is divided into two by a transverse chitinous bridge (Fig. 42, right sub-median wall chit. br.). Each sub-ventral wall of the mouth capsule has a transverse furrow running through the middle of its length, and is curved anteriorly and posteriorly to the same furrow. The transverse furrow and the two curves are seen in longitudinal section in Fig. 42 in the right sub-ventral wall (chit. arc. 1 and chit. arc. 2). The same details are shown in frontal projection in the left sub-ventral wall. The dorsal wall of the mouth shows the anterior curvature thicker and longer than the sub-ventral one (chit. arc. 1 dorsal wall). On longitudinal section it has the shape of the letter S. Its caudal end continues into the dorsal lining of the lumen of the œsophagus (Fig. 42, chit. arc. 2). The dorsal wall is divided along the longitudinal median axis by a deep cleft. At the posterior end of the cleft the buccal lancet is inserted. In Fig. 42 the left edge of the above cleft is represented (chit. arc. 1 dorsal wall).

Buccal Lancet.—The buccal lancet has a bilobular base, a pointed tip, and is compressed laterally like the blade of a knife. The dorsal edge is conspicuously curved, and has two thorn-shaped points, of which the more anterior one is the sharper (Fig. 42). The lancet is connected with the mouth capsule by an articulation which allows an oscillatory movement in a dorso-ventral direction. Two muscles, which may be called the muscles of the lancet, are inserted on its base. The more anterior one is the stronger (Fig. 42 m. abd. lan.). It is inserted on the antero-dorsal lobe of the base of the lancet, from which it takes a dorsal direction, through the muscular fibres of the œsophagus, and ends apparently on the external surface of the œsophagus. This muscle is seen with difficulty in fixed

and cleared specimens, but it is quite distinct in fresh specimens, especially when the worm is still alive, and is moving the lancet. I call this muscle the "abductor of the lancet." The second muscle is inserted on the postero-ventral lobe of the base of the lancet, from where it is directed posteriorly, and after passing a comparatively long distance through the muscular walls of the œsophagus, it ends in the marginal portion of the œsophagus wall (Fig. 42). This muscle is less powerful than the first one, and is apparently compressed laterally. I call it the "adductor of the lancet" to distinguish its function from that of the former.

Function of the Mouth.—In observing a live worm under strong magnification, it is possible to see rather complex and co-ordinated movements of the different parts of the mouth apparatus, of which there are four movements which are typical.

- (1) Periodical movements of the lips, resulting in changes of the mouth opening.
- (2) Anterior projection of the lancet.
- (3) Very frequent oscillatory movements of the lancet in the ventro-dorsal direction.
- (4) Some decided and marked jerks of the head in a ventro-dorsal direction.

The movements of dilatation of the lips appear to be produced by the cephalo-œsophageal muscles, which take insertion on the base of the lips. In connecting the movements of the mouth with its conformation, it is allowable to conclude that the lancet first pierces the mucosa of the stomach, the hooked point of the dorsal margin then lacerating the tissues as it is being withdrawn. The occasional jerks of the head have apparently also the purpose of lacerating the tissue. It could also be mentioned that the muscular walls of the œsophagus are sometimes seen in progressive contraction antero-posteriorly. In examining the intestinal contents of a *Haemonchus contortus*, it seems that small particles of the mucosa of the stomach are also ingested.

Esophagus.—The œsophagus is club-shaped, about 1.5 mm. long, with a diameter of 30μ at the anterior end and 150μ at the base. The lumen is tri-radiate when contracted (Figs. 43–44). Posteriorly the lumen ends in a short triangular cavity, the walls of the cavity forming three lips. The three lips represent the three œsophageal valves, very similar to those described in other strongylidae. The walls of the œsophagus are thick and muscular. Two portions of muscles can be distinguished, namely, the marginal portion, consisting of fibres arranged in three bundles, each one starting from the triangular edge bordering the lumen and diverging towards the periphery (Fig. 45, fbr., marg.), and the second portion representing the muscles proper of the œsophagus, radiating from the centre and in cross section appearing to be divided into three segments (Fig. 44, mu. aes.). Within the muscles are included the three œsophageal glands. The muscular fibres run at right angles to the axis of the œsophagus except for a short distance in the posterior part, where they are more obliquely disposed, and converge anteriorly. This tract only extends to within 40μ or 50μ of the end of the œsophagus, in which portion the muscles are again arranged

at right angles to the axis, and now represent the muscles of the œsophageal valves.

Esophageal Glands.—The œsophageal glands number three, each occupying a section of the muscular wall of the œsophagus. The dorsal œsophageal gland is the largest, and is seen on cross section to reach the anterior tip of the œsophagus (Fig. 43, gl. dors.). The two sub-ventral glands start anteriorly at the level of the cervical papillae. The œsophageal glands continue along the œsophagus, and towards the posterior end split into two, turning completely round in the form of two hooks. The two branches proceed anteriorly as far as the cervical papillae (Fig. 45, gl. ocs. sub.). The nuclei of each œsophageal gland can easily be detected in cleared specimens where the œsophageal gland turns anteriorly, having a diameter of about 14μ . The efferent duct of the sub-ventral glands can be seen clearly outlined for a good distance, extending into the median trunk of each gland, until at about 95μ – 100μ from the anterior tip of the same trunk, the efferent duct turns off and opens into the œsophageal lumen. This opening is situated in the first half of the length of the œsophageal lumen, rather far back. The efferent duct of the dorsal gland passes from the anterior tip of the œsophagus to the dorsal lip of the mouth, and opens into the mouth cavity in the cleft in which the lancet is lodged.

Intestinal Valves.—The valvular apparatus between the œsophagus and the chyle intestine appears as a thick short cylinder about 47μ in length and 67μ in diameter, projecting into the lumen of the chyle intestine, and showing on its outer surface a circular sulcus. This sulcus divides the cylinder into the two rings already noted in the larval stage. The anterior ring has a fibrous appearance, surrounding the end of the œsophagus and connecting it with the outer wall of the chyle intestine. The second one protrudes into the intestinal lumen, and is characterized by its granular appearance.

Chyle Intestine.—The chyle intestine is 24–26 mm. long in the female, and 15–16 mm. in the male. In the female, from the œsophagus to the tip of the ovarian tube, it is loosely attached to the walls of the cavity. For two-thirds of its length from the œsophagus to the vulva, the chyle intestine and the genital tubes run parallel in a spiral. The thick spiral formed by the two organs occupies practically all the lumen of the coelomic cavity. At the level of the vulva, the intestine lies ventrally until it reaches the rectum. In cross sections of a female worm, the intestine appears round in the portion situated anteriorly to the ovary. At the level of the first portion of the ovaries it is semi-circular, about 228μ by 120μ , with a large lumen. Along the ovarian tube the intestine is more compressed (Fig. 51, int.). At the level of the ovijector and vagina, it is again more circular in shape, and the diameter measures 160μ by 120μ (Fig. 56, int.). Posteriorly to the vulva the intestine is semi-circular in shape. In the last portion it is still 214μ by 124μ , with thick walls rich in nuclei. In the male, at the level of the seminal vesicle, the chyle intestine is crescent shaped on section (Fig. 46, int.). Along the cement gland the intestine is compressed, and still crescent shaped on section. The lumen is sometimes S-shaped (Fig. 47, int.). The dimensions are

about 173μ as a maximum diameter, and 80μ in the final portion of the intestine.

In conclusion it may be stated that in both male and female, the intestine is very plastic, and adapts itself to the disposition of the neighbouring organs. The cells composing the walls of the intestine are in both sexes more or less filled with granulations. In young worms the outlines of the chyle cells can sometimes be detected. The plasma granulations are more or less mixed with pigment, being coarser and more yellowish in the younger worms, and more abundant and black in colour in older specimens, and it appears on cross section that when the intestine is crescent shaped, the black granulations are more often found at the tip (Figs. 46-53, int.). The nuclei of the above cells are of two kinds, namely, a larger nucleus irregularly shaped, which is not so commonly found, and more commonly a smaller circular nucleus with a diameter of 15μ . The internal lining is cuticular in appearance, about 8μ in thickness, with vertical striations and a smooth surface.

Rectum of the Male.—In the male the rectum appears as a cone protruding into the bottom of the cloaca, and measures about 50μ in length, with a base diameter of 40μ . The lumen is $3-4\mu$ broad, and the lining appears chitinous, and is differentiated from the lining of the chyle intestine, which is always more brownish in colour. The chyle intestine is connected with the rectum by a thick granular band called the "anterior ring of the rectal ligament." It gives origin to three cells. Two of these cells have a diameter of $45-50\mu$, and are situated on the latero-ventral side of the rectum. The third one is dorsally situated. From the anterior rectal ring, a thin strand proceeds backwards for a short distance along the walls of the cloaca, bounded posteriorly by a second less conspicuous ring called the "posterior ring of the rectal ligament." From this ring three cells of about 15μ in diameter protrude ventrally. The "rectal sphincter" is represented by a muscular band which can be observed anterior to the anterior ring of the rectal ligament.

Rectum of the Female.—The rectum in the female consists of a chitinous tube about 0.117μ in length. The anterior portion is connected with the chyle intestine, and appears funnel shaped, similar to that described in the earlier stages (Fig. 58). The length of this part is about 50μ , with a width of 20μ at the base; its connection with the chyle intestine is distinct. The succeeding portion of the rectum has a diameter of a few microns only, and runs nearly parallel to the ventral wall of the worm (Fig. 58, rect.). The lining of the rectum is chitinous, and thicker on the dorsal side of the second portion, where the maximum thickness of 14μ is registered. At the level of the anus the internal lining is quite distinct from the neighbouring skin cuticle. The pulvillus post analis, already noted in other stages, is now well marked. It consists of two portions, the anterior granular portion, with an average length of 80μ , and the posterior portion continuing to the tip of the tail (Fig. 58, pulv. p.a.). The anterior ring of the rectal ligament, as well as the thinner posterior portion extending on the walls of the rectum, are conspicuous (Figs. 58-59, lig. rect.). The posterior ring of the rectal ligament is covered by the pulvillus post analis. Both anterior and posterior rings show the three cells already mentioned in the male. The rectal

sphincter is seen on the edge of the anterior ring of the rectal ligament. From the dorsal side of the posterior rectal portion, a strand of muscular cells radiate dorsally with a fan shaped arrangement (the so-called "anal muscle") (Fig. 58, m. an.).

Genital Organs of the Male.

The genital tube is well developed and occupies the greater portion of the body cavity. In a full-grown male of 17 mm. in length the sexual tube is 11 mm. in length, varying in diameter in different parts of the body. The main portion of the sexual tube runs ventrally, but the anterior end can be found twisted round the intestine. The vesicula seminalis is sometimes seen dorsally or on the lateral side.

Testes.—The testes are the part of the tube bounded posteriorly by the vesicula seminalis. They are either slightly twisted around the chyle intestine or are stretched. The anterior end just fails to reach the level of the nucleus of the right cervical gland by a few microns. The length of the testes tube is about 4–5 mm. The external configuration of the anterior end is conical, and the diameter of the tube varies somewhat according to its position with the chyle intestine, but has an average diameter of 100μ in the anterior and 150μ in the posterior part. In examining a whole specimen its structure appears finely granular. On cross section the internal structure shows a layer of conically shaped cells disposed radially at right angles to the axis, leaving a central space occupied by round cells. More posteriorly the layer of peripheral cells can still be seen, and in the median axis runs a canal with a diameter of 4μ . The more posterior part of the testes is full of spermatozoa (Fig. 40, te.).

Vesicula Seminalis.—This reservoir constitutes the posterior continuation of the testes and is frequently bottle shaped, with the base being situated anteriorly (Fig. 40, vs.). It is connected with the testes by the testicular canal about 200–250 μ in length by 57μ in diameter. This canal is always coiled so that the two tips of the testes and the vesicula seminalis almost touch each other. Posteriorly the vesicula seminalis is connected with the cement gland by the "vesicular canal," having a length of 140 μ and a diameter of 40μ . The vesicula seminalis varies between 350 μ and 700 μ in length. In cross section the thicker part of the vesicula seminalis is either oval or semi-circular in shape. The walls are thin and the seminal reservoir is full of spermatozoa (Fig. 46, vs.). There is a close similarity in the structure of the last portion of the testes, seminal duct, and vesicula spermatica, and the vesicula seminalis can be recognized as such by a simple constriction separating it from the posterior end of the seminal duct.

Spermatozoa.—The spermatozoa contained in the vesicula seminalis are small, spindle shaped bodies of about 3–5 μ in length by 1.5 μ thick, and consists of a strongly refragent substance. Each spermatozoon is surrounded by a mass of very finely granular protoplasm, spherical in shape and about 5–6 μ in diameter (Fig. 46, vs.).

Cement Gland.—The cement gland is the portion of the sexual tube connecting the vesicula seminalis with the cloaca. The following descriptions were taken from a specimen in which the length of the cement gland measured

6.85 mm. In its essential structure it consists of a canal with a comparatively thin lumen, and thick walls composed of two longitudinal lateral rows of cells. It is possible to distinguish two portions of the cement gland, the anterior and the posterior one, separated from each other by a slight constriction.

Anterior Portion.—The anterior portion measures about 4 mm. in length. From the external appearance the two rows of cells are flattened antero-posteriorly, slightly obliquely to the main axis of the canal (Fig. 40, gl. cem. 1). The cross section of the same portion appears slightly compressed dorso-ventrally. The lumen is nearly linear, and disposed in the direction of the shorter diameter of the section. The cells of the two lateral rows appear intact contrary to the appearance of these cells in the second portion of the gland.

Posterior Portion.—The posterior portion of the cement gland is about 3 mm. in length, passing posteriorly through the ligamentum rectalis, where it is reduced to a thin chitinous canal ending in the cloaca. Seen externally the cells of each lateral row are thin, sometimes hardly distinguishable from each other and placed more obliquely to the longitudinal axis of the tube than in the anterior portion of the gland. This arrangement has the appearance of the lateral barbs of a feather (Fig. 40, gl. cem. 2). On cross section the periphery of the tube resembles the anterior portion, but the inner portion of the cells protruding into the lumen of the canal is broken off and the base of the cell only remains (Fig. 47, gl. cem.). It appears that the cement is produced by the successive breaking down of the row of cells of the second portion, commencing with the cells nearer the efferent duct and proceeding anteriorly. The anterior part of the gland where the cells are still apparently intact would represent the portion not yet used.

Ano-genital Aperture.—It consists of a transverse slit opening in the ventral part of the floor of the bursa. It possesses a dorsal and a ventral lip. The dorsal lip is supported by a prominence of the floor of the bursa gradually rising from its dorsal edge and reaching a level above the dorsal lip. The ventral lip is supported by the genital cone. The genital cone rises for 70–75 μ above the floor of the bursa with a base of 150 μ . The walls of the genital cone are divided into three sections by two anular constrictions. The tip is reduced to a thin appendix of finger like appearance (Fig. 60, ge. co.). The pulp of the cone is a direct continuation of the ventral band. On each commissure of the ano-genital opening is present a small pyramidal protuberance, rising from the floor of the bursa. The two appendages with the above-mentioned supports of the two lips join to form a funnel shaped cavity, at the bottom of which is the ano-genital opening.

Cloaca.—This is an elongated cavity measuring about 70–85 μ in length. The anterior end is larger than the posterior one and receives the opening of the rectum, of the ejaculatory duct, and of the spicular canal. The dorsal wall has a deep cleft in which the spicules are seen when exerted. The ventral wall is formed by the ventral band. The posterior end corresponds to the ano-genital aperture.

Spicular Apparatus.—There are two spicules placed dorsally to the posterior part of the intestine and rectum. They have the appearance of a horn 460–470 μ long, slightly twisted, of brownish golden colour. The tip of each spicule is at the posterior end, and shows a small knob about 12 μ in transversal diameter. At a distance of 20 μ for the left spicule and at a distance of 40 μ for the right spicule measured from the tip, a barb projects forwards. At a distance of 120 μ from the tip the median surface of each spicule is flat, gradually widening out until it reaches 16–20 μ in width. The borders are thick at the flattened parts and converge towards one another as thin bands (Fig. 47, spic.). The last part of the spicular cavity contains a granular substance called by Looss “Pulp of the Spicule.” Amongst it some granulations appear, resembling the cement granulations, very refragent, and with a diameter of .3–.5 μ . Protruding from the cavity of the spicule a capsule or bag can be seen extending anteriorly, measuring 200–250 μ long and 70 μ broad, the walls being composed of two layers, of which the internal and more refragent one is 11–12 μ thick. “Pulpa spicularis” is also contained in this capsule. The “retractor muscles” of the spicule are 28 μ broad, proceed anteriorly along the walls of the intestine and finally are connected with the lateral bands, about 450 μ from the base of the spicule. The “extor muscle” is sometimes seen, in a whole specimen, proceeding backwards along the walls of the spicule.

Spicular Canal.—The spicular canal is chitinous and protrudes into the cloaca dorsally to the rectum; its first portion consists of a simple canal. At the anterior end of the gubernaculum this canal bifurcates and at this level it is compressed dorso-ventrally, measuring about 70 μ by 30 μ in diameter. Proceeding further anteriorly the two bifurcations of the spicular canal are completely separated (Fig. 47, can. spic.). They can be followed for some distance whilst diverging laterally, but the walls become thinner until they can no longer be seen.

Gubernaculum.—From both sides of the posterior portion of the spicular canal are chitinous appendages which converge dorsally and medially to meet each other. Where the two branches meet, they give rise to the gubernaculum (Fig. 47, can. spic.). In a whole specimen the gubernaculum has the appearance of a date stone, measuring about 200–250 μ in length and 35–40 μ in width. The margins are curved dorsally, forming a deep longitudinal furrow (Fig. 47, gub.). The substance of the gubernaculum has the same appearance as that of the spicules. The two most prominent muscles of the gubernaculum detectable in cross section are:—

- (1) The Musculi Seducor Gubernaculi inserted into both sides of the gubernaculum and crossing the body cavity, proceeding to the lateral bands (Fig. 47, m. sed. gub.), and
- (2) The Musculi Supinator Gubernaculi. Portions of this muscle appear in cross section inserted in the dorsal part of the gubernaculum, from where they diverge towards the base of the bursa (Fig. 47, m. sup. gub.).

Bursal Apparatus.—The bursa of *Haemonchus contortus* consists of two symmetrical lateral lobes and an asymmetrical dorsal one. Each

lateral lobe starts from the posterior end of the body as a trunk with a diameter of 150μ (Fig. 49, tru. 1, lob.). The membrane of the lobe starts dorsally near the floor of the bursa, and ventrally at the base of the genital cone (Fig. 48, ge. co.). The dorsal and ventral origin of the membrane is shown in cross section in Fig. 49 (ma. dors. and ma. ven.). The appearance of a lateral lobe when distended is that of a roughly rectangular leaf with the posterior end convex (Fig. 48). On cross section the disposition of a lateral lobe is semi-circular (Figs. 49-50), with the posterior part slightly turned inwards (Figs. 40 and 60). On the external surface the lateral lobes are transversely striated (Fig. 50, cut. ext.). At a short distance from the margin of the bursa the transversal striations bifurcate. Two papillae are found in the external surface of the lateral lobe, corresponding to the tip of the extero-dorsal and extero-lateral rays. The inner surface of a lateral lobe has first a rather deep and narrow groove originating at the base of the genital cone. It runs rather close and parallel to the ventral margin until it crosses the ventro-ventral ray. From this point the groove turns in a median direction, and with its two branches ends on the medio-lateral ray (Figs. 48, 49, 50, int. gr.). I call this groove the "internal groove of the lateral lobe." The outer side of the groove is bordered by a rather large corrugated ridge (Figs. 48, 50, rid. int. gr.), which in the more posterior course branches towards the posterior margin of the lobe. The inner border of the internal groove is also rather prominent and can be easily seen in sections (Fig. 50, rid. int. gr.). The second prominent structure of the medial surface of the lateral lobe is a finely lobulated protuberance ending with the anterior tip between the postero- and medio-lateral rays at the level of the tip of the extero-dorsal ray, increasing forward in width and decreasing in thickness till it reaches the internal groove. This structure is called in the present paper the "internal gibbosity of the lateral lobe," and is seen in frontal view in Fig. 48 (int. gib.), and in section is represented by the morula-like protuberances at the level of the three lateral rays in Fig. 50. Some cuticular tubercles also appear on the posterior portion of the three lateral rays. Along the margin of the lobe the cuticular layer shows a very fine striation corresponding to a similar disposition seen on the external surface, giving the margin of the lobe a denticulated appearance (Fig. 48). Four papillae are also seen in the inner surface of the lateral lobe corresponding to the tip of the postero and medio-lateral rays and to the tip of the latero and ventro-ventral rays. The already mentioned corrugated structure of the medial surface of the lateral lobe is peculiarly adapted to increase the adhesive power of the bursa in gripping the female.

Substance of the lateral lobes of the bursa.—The bursa appears composed of an external and internal cuticular layer, between which is contained a single thick subcuticular layer. The external cuticular layer is also rather thick and in direct continuity with the cuticle of the body (Fig. 50 cut. ext.). The internal cuticular layer is not always distinct on account of the tubercles already described as occurring in the medial surface of the lateral lobe. The subcuticular layer is rather coarse grained where the so-called "cuticular granules" are very numerous and large (Figs. 48, 49, 50, gran. cut.). In the subcuticular layer are the rays of the bursa.

The rays in the transversal section appear round or oval with a proper envelope. In the internal cavity appears a granular substance (Fig. 48, pul. ray.), a rather thick muscular coat formed by the costal muscles (Figs. 49-50), and a nervous fibre called "Costal nerve" (Figs. 49-50, nrv. co.).

Disposition of the rays in the lateral lobe of the bursa.—At about 50μ from its origin, the common trunk of the rays gives off the *externo-dorsal ray*. This ray diverges slightly from the common trunk, running straight to the dorsal margin of the lobe. After a distance of about 500μ it ends, at 28μ from the dorsal margin, in a papilla situated on the external surface of the lobe. It is the thinnest of the rays of the lateral lobe, and slightly decreases in diameter towards the tip (Figs. 48, 49, 50, ex. drs. r.). At about 180μ from its origin the common trunk divides into the trunk of the lateral rays and the trunk of the ventral rays. The trunk of the lateral rays proceeds undivided along the median axis of the lobe for about 100μ , where dorsally it gives off the postero-lateral ray. After another 70μ it divides into the medio-lateral and externo-lateral ray. The *externo-lateral ray* appears like the continuation of the common trunk of the lateral rays and runs along the median axis of the lobe, ending at about 40μ from the posterior margin of the lobe in a papilla situated on the external surface of the lobe. It is the thickest of the lateral rays, about 470μ in length and about 57μ in breadth at the base (Figs. 48, 50, ex. l. r.). The *medio-lateral ray* runs close to the externo-lateral one for a short distance, and afterwards curves towards the dorsal margin of the lobe, where it ends in a papilla situated on the inner face of the lobe at about 15μ from its margin. It is about 456μ long by 50μ thick at the base (Figs. 48-50, md. l. r.). The *postero-lateral ray* runs very close and nearly parallel to the medio-lateral ray. It is about 530μ long by 35μ thick on the base, ending at 10μ from the dorsal margin of the lobe in a papilla situated on the internal face of the lobe (Fig. 48, ps. l. r.). The common trunk of the ventral rays runs undivided and close to the externo-lateral ray for about 140μ (Fig. 48, ven. r.), and divides into the latero-ventral and ventro-ventral ray. The *latero-ventral rays* runs for a short distance with the externo-lateral ray and then curves suddenly towards the point of division between the dorsal and posterior margin of the lobe (Fig. 48). It is about 371μ long, 35μ thick on the base, and ends at about 15μ from the margin in a papilla situated on the internal surface of the lobe (Figs. 48-50, lat. ven. r.). The *ventro-ventral ray* is the shortest ray of the lateral lobe, diverging suddenly from the latero-ventral ray towards the ventral margin, where it ends about 7μ distant in a papilla situated on the inner surface of the lobe. It is 192μ long by 22μ thick on the base (Figs. 48-50, ven. ven. r.).

Asymmetrical dorsal lobe of the bursa.—This lobe takes origin on the internal surface of the left lateral lobe and at the base on the dorsal margin of the internal gibbosity. It is directed medially and slightly dorsally to the main axis of the body. Its shape is roughly quadrangular, about 160μ broad by 150μ long. The margin is irregularly lobulated (Fig. 48, drs. lb.). The root is thick and nearly round, with coarse cuticular granules (Fig. 49, drs. lb.). The dorsal ray takes origin from the common trunk of the lateral rays near the origin of the externo-dorsal ray. On

cross section it is slightly oval (Fig. 49, drs. r.). The dorsal ray runs undivided along the axis of the dorsal lobe for about half its length, then bifurcates into two equal thin branches running to the corners of the lobe, and ends in two very small secondary branches. The external branch ends in a small papilla on the inner surface of the lobe. The median one protrudes for some microns through the margin of the lobe (Fig. 48, drs. r.) and forms a small pointed hook.

Genital Organs of the Female.

Position of the Genital Opening.—The genital opening, or vulva, is situated in the ventral side of the body at about one-fifth or one-sixth of the body length from the tip of the tail. The following are the measurements of six worms, showing the exact place at which the vulva was situated:—

(1)	Total length of worm	18 mm.,	vulva situated	3.5 mm. distant.
(2)	„	20 mm.,	„	3.4 mm. „
(3)	„	24 mm.,	„	4.5 mm. „
(4)	„	27 mm.,	„	5 mm. „
(5)	„	30 mm.,	„	5.5 mm. „
(6)	„	33 mm.,	„	5.5 mm. „

The anterior genital tube commences at the vulva, and as a rule proceeds obliquely to the dorso-lateral side of the intestines and maintains that position for the length of the uterus (Fig. 52). In other cases the anterior uterus crosses the intestinal tube dorsally and descends in the opposite side (Fig. 41, ant. ut.), where it makes a large loop before assuming the spiral shaped disposition. The posterior genital tube proceeds along the latero-ventral side of the body for the whole length of the uterus (Fig. 52). The following ovarian tube turns sharply in front of the anus and runs ventrally again until it reaches the level of the genital opening. It then crosses the genital apparatus and the chyle intestine dorsally (Fig. 41), turning again ventrally along the right side of the anterior uterus, and then coiling around the chyle intestine together with the anterior ovarian tube (Fig. 41). Altogether the two ovarian tubes make 12–14 turns around the intestine, developing a length of 23–25 mm. They end at about the level of the nucleus of the right cervical gland, that is to say 15–16 mm. from the vulva in an average sized specimen. The posterior ovarian tube is 3–4 mm. longer by reason of its origin. The tip of the anterior ovarian tube is usually a short distance in front of the tip of the posterior ovarian tube (Fig. 41).

In old worms the anterior end of one or both of the ovarian tubes turns backwards, making another turn around the intestine, so that the transverse section of this portion may show four ovarian tubes instead of two. The diameter of the tubes decreases slightly from the place of origin. The average thickness of the anterior tube can be taken as 135μ in the posterior end, and 75μ in the anterior portion. In each coil the thinner of the two tubes is usually the posterior one (Fig. 51). The following notes on the different sections of the genital apparatus refer to the anterior tube.

Structure of the Ovaries and Oviduct.—The anterior portion of the ovarian tube appears as a canal with thin transparent walls. It contains

the ovarian cells grouped radially around a granular medial axis (rachis), and directed in an oblique direction, resembling a fir cone. The rachis with the adhering ovarian cells is easily observed in dissecting out the anterior portion of the ovarian tubes from a fresh worm. The rachis is seen protruding from the canal for a long distance with the eggs attached by a short thin peduncle proceeding backwards. The ovarian cells are about $10\text{--}15\mu$ thick, flat antero-posteriorly, and in a transversal section of the ovarian tube they are seen occupying about one-third of the circumference. They consist of a thin membrane with granular contents, and possess a large spherical nucleus. Proceeding further backwards the rachis disappears, and the eggs increase in size and become irregular in shape owing to compression by the other eggs. They are free in the ovarian tube (Fig. 51, ant. ov.; Fig. 53, post. ov.) For the last 3 mm., the oviduct decreases in diameter until it reaches a distance 35μ from the end, where the walls appear thicker. Eggs are seen in this portion placed in a long row, sometimes close together and at other times separated from each other.

Uterus.—The uterus is about 2.25 mm. long, and is generally spindle shaped. The thickness varies according to the number of eggs present in its cavity (Fig. 41, ant. ut.; Fig. 53, ant. ut.). The walls of the uterus are thin and no external muscular layer is seen (Fig. 53, ant. ut.). The epithelial layer, representing the internal lining, consists of rhomboidal cells disposed in circular rows (Fig. 52, post. ut.). In the distended part of the uterus the epithelial cells are rather thin. The anterior 8–10 rows of epithelial cells protrude conspicuously into the lumen of the uterus, taking a disposition similar to the leaves of an onion. In the posterior portion of the uterus the epithelial cells are rather thick and give the walls of the lumen a rough surface (Fig. 52, ant. ut.). This part of the uterus contains the greatest number of spermatozoa of any part of the genital tube. It seems to correspond to the “Receptaculum seminis” recorded by Looss in the *Ankylostoma Duodenale*.

Spermatozoa.—The spermatozoa found in the seminal receptacle are similar to those found in the seminal vesicle of the male. When the seminal receptacle is dissected and the spermatozoa on the slide come in contact with water, the surrounding protoplasmic sphere is broken and the spermatozoa resemble small bacteria, very refrangent, and capable of marked motility.

Ovijector: Pars haustrix.—The length is about 350μ , with an anterior diameter of 150μ and posterior of $170\text{--}175\mu$. The inner walls are formed anteriorly by four large cells arranged longitudinally in pairs, and having the appearance of a petal of a calix (Fig. 52, pars h. 1). Posteriorly the above four cells are followed by four smaller ones, protruding into the lumen of the pars ejectrix like a plug (Fig. 52, pars h. 2). The external muscular layer of the pars haustrix is comparatively thin and transversely striated (Figs. 52 and 54, m.p.h.).

Pars ejectrix.—The pars ejectrix is roughly cylindrical in shape. It is placed in the left ventral side of the body in front of the vulva. In length it measures 215μ . The more anterior portion is formed into a

sphincter, and appears like a short bulb; the muscular walls are very thick and transversely striated. The diameter of this section measures 146μ (Fig. 52, pars ejec., 1). The rest of the pars ejectrix is about 150μ thick, with the muscular walls thinner than the anterior portion and longitudinally striated (Fig. 52, pars ejec. 2). The inner walls of the pars ejectrix show numerous folds separated by longitudinal and transversal furrows (Fig. 52, pars ejec. 2; Fig. 55, int. l.p. ejec.).

Unpaired Part.—The unpaired tube joins the proximal ends of the posterior and anterior ovijector, and as a consequence is situated rather transversely to the main axis, proceeding from the left ventral to the left lateral side (Fig. 52, u. pars.). In length it is about 210μ , the ventral wall being nearly straight, whilst the dorsal wall is convex. In the centre its diameter is 112μ and at the end 80μ . The external layer is granular and $5-6\mu$ thick (Fig. 56, u. pars.). The muscular wall is about 11μ thick with longitudinal striations (Fig. 52, u. pars.). At the point of connection with the pars ejectrix, a marked constriction is noted, but the muscular fibres are not broken. Between the muscular layer and the inner lining a cuticular wall can be seen, with a thickness of about $6-8\mu$. The internal lining is longitudinally folded (Fig. 52, u. pars.). In this unpaired part, three or four eggs are frequently present during the period of oviposition. In the ventral wall and in the centre, a circular opening with a diameter of $35-40\mu$, surrounded by a strong sphincter, gives entrance to the vagina. Other authors dealing with different worms of similar structure assume that this portion of the genital canal belongs to the pars ejectrix, but the distinct function and sharp external delimitation from the two opposite ejectrix organs and from the vagina would suggest the advisability of considering this unpaired tube in the *Haemonchus contortus* as a vestibule between the vagina and the two paired genital tubes.

Vagina.—The vagina is a short tube joining the unpaired tube with the vulva (Fig. 52, vag.). It is 150μ long by $80-85\mu$ in diameter, externally. The lumen undergoes dilatation and contraction. Conspicuous and numerous muscular fibres, in bundles, start perpendicularly from the walls and radiate into the granular mass of the linguiform process (Figs. 52 and 56, m. vag.). The walls are cuticular, $6-8\mu$ thick. The internal lining shows numerous longitudinal folds. The posterior end reaches the linguiform process on the base of its medial wall (Figs. 52 and 56, m. vag.).

Vulva.—The vulval opening is situated at the base of the linguiform process (Fig. 52). It possesses conspicuous cuticular lips protruding outside perpendicularly to the axis of the linguiform process, and consequently towards the axis of the body. These lips form a slight tube compressed antero-posteriorly. The anterior wall is flat and the posterior wall slightly curved (Fig. 52, l. vulv.). When the lips are pressed against the body by the linguiform process, the vulva remains closed.

Physiology of the Ovijector.—In observing a living female worm under fairly high magnification, the irritation of manipulation causes the female to lay eggs, so that it is quite easy to observe the mechanism of this process. The eggs are passed out singly or a few at a time with intervals in between. One ovijector is used at a time. In considering for instance the anterior

ovijector, the following mechanism can be observed:—The pars haustrix is projected anteriorly, and at the same time opens (Fig. 52, pars, h. 1). By this movement two or three eggs are engulfed in it. Immediately afterwards the pars haustrix returns to its original position and the eggs are pushed into the pars ejectrix. The marked constriction of the anterior part of the pars ejectrix (Fig. 52, pars ejec. 1) is now followed by a gradual contraction of the subsequent fibres and the eggs are pushed into the vestibule (Fig. 52, u. pars). Here other eggs are usually present, and by the pressure of the oncoming ones they are pushed through the vagina and the vulval opening. The function of the vestibule is a passive one, except for the slight pressure exerted by the dorsal wall, by which the egg is diverted ventrally to fall into the vagina. The dilatation and the sudden contraction of the vulval opening after the expulsion of the eggs is very marked. Briefly, it can be said that the mechanical act of laying eggs consists of two distinct processes. In the first place the pars haustrix receives the eggs from the uterus and transmits them to the subsequent portion of the tube. In the second process undertaken exclusively by the pars ejectrix, the eggs are pushed through the vestibule of the vagina into the vulval opening.

Linguiform Process of the Vulva.—This is very conspicuous, commencing just in front of the vulva and extending backwards in a slightly oblique direction (Fig. 52, ling. proc.). Its length is about 750μ by 250μ . At the base it is compressed dorso-ventrally. The internal side is slightly concave, whilst the external side is convex (Fig. 56, lin. proc.). The chitinous mantle is very thick and shows transverse striations on the surface. The internal part of the linguiform process has a granular appearance, and is a deviation of the ventral band. The cuticular granules are sometimes very numerous (Fig. 56, gran. cut.). The structure of the linguiform process and its temporary changes suggest that it is an organ erectile during copulation.

Lateral Vesicles.—At the time of copulation, there is present, on one or both sides of the body at about the level of the base of the linguiform process, a more or less conspicuous cuticular vesicle varying in diameter from 50 – 350μ (Fig. 56, lat. ves.). The base of this vesicle is usually smaller and more laterally compressed like a peduncle. Judging by section, these vesicles seem to be formed by a simple swelling of the external cuticular layer at the level of the lateral bands. The walls are transparent and show marked transverse striations. Sometimes they contain a granular substance, spreading like a fan from the base. At other times the vesicle seems empty. During copulation the lateral vesicles are usually covered by the lateral lobes of the bursa of the male. The predominant appearance of these vesicles at the time of copulation, their temporary disappearance, and their varying numbers, indicate that they are accessory organs for the copulatory act.

Apparent Anomalies of the Vulval Linguiform Process.—In general I found that the anatomy of *Haemonchus contortus* was fairly constant in regard to appearance and size of the different organs, but at the time of marked reproductive activity I met with a very large number of rather old female worms, showing peculiar differences in the linguiform process.

In some sheep about 25 per cent. female worms showed the following peculiarities:—The linguiform process was unusually short, measuring 250μ in length and about 170μ at the base. It was conical in shape, adhering to the body and slanting towards the tip, with contents granular in appearance. In other specimens it was represented by a pimple-like body, protruding for a distance of about 25μ , sometimes placed anteriorly and at other times situated laterally to the vulva. In some specimens the linguiform process was quite absent and the opening of the vulva was only indicated by a rudiment of the above-mentioned vulval lips. There were no other remarkable changes in the remainder of the genital organs except that the vagina was sometimes situated in a direction perpendicular to the ventral side of the body, instead of occupying the oblique position already described. The laying of eggs was performed quite normally. I might also add that the anomalies mentioned were found in different seasons of the year.

Nervous System.

The nervous system of *Haemonchus contortus* very closely resembles that described in *Ascaris megaloccephala* and *lumbricoides* by Leukart, and in *Ankylostoma duodenale* by Looss. The nomenclature and the method of investigation of Looss constitute an advance in our knowledge of the anatomy of the nervous system of nematodes. I have therefore adopted them, and the following notes are intended chiefly as a comparison between the nervous system of *Haemonchus contortus* and that of *Ankylostoma duodenale*. The corresponding drawings, as also the notes themselves, were made from specimens cleared in glycerine. Comparatively young worms were selected as being more transparent and less pigmented. The males were 12 mm., in length and the female specimens 17 mm. For the more minute details a magnification of 1334 diameters was used.

Central Nervous System.—The central nervous system appears to be composed of the “nerve ring,” the “cephalic ventral ganglion,” two “cephalic lateral ganglia,” and the cephalic “anterior commissure.”

Nerve Ring (Cephalic Commissure).—In the male the nerve ring is situated about 270μ , and in the female 285μ , from the anterior end of the body. In lateral view it has a slightly oblique direction antero-posteriorly from the dorsal to the ventral side. In dorso and ventral view it is situated transversely to the body, and measures about 22μ in thickness (Fig. 57, co. ceph.). When seen in cross section it occupies the larger part of the coelomic cavity around the oesophagus (Fig. 44, co. ceph.). It appears to be composed of very fine fibres arranged in bundles, having a slightly wavy appearance. Only a few nucleated cells are present amongst the fibres of the nerve ring. In the latero-ventral view the superficial nerve fibres are seen to take a more oblique direction than the inner ones, and proceed from the lateral to the ventral cephalic ganglion (Fig. 57, sup. f.). This peculiar disposition of the two layers of fibres results in the formation of a small area in the ventral side of the nerve ring, where nerve fibres are very rare or completely absent (Fig. 57).

As stated above in dealing with the “subcuticle” the four subcuticular longitudinal bands run on the periphery of the nerve, acting as

a kind of support. The dorsal band bifurcates at the nerve ring, forming two lateral bridges (Fig. 44). The lateral bands are also bifurcated, and of these two bridges the upper branch is connected with the lateral adventitious cell. The ventral band running alongside the nerve ring is connected on both sides with the two ventral adventitious cells.

Cephalic Ventral Ganglion.—In ventral or latero-ventral position a certain number of pyriform nerve cells are seen just under the ventral band, and projecting backwards behind the nerve ring (Fig. 57, ggl. ceph. v.). This group of cells represents the "cephalic ventral ganglion." It is horse-shoe shaped, with the five more anterior cells adhering to the posterior margin of the nerve ring, and the two branches, each composed of ten or eleven cells, directed backwards and slightly diverging on to both sides of the excretory pore. Of the five anterior cells, the three middle ones are more developed, the central one being the largest, 11μ long by 8.4μ thick, and situated more superficially. This arrangement closely corresponds to that described by Looss in the Cephalic Ventral Ganglion of *Ankylostoma duodenale*.

The Cephalic Lateral Ganglia.—This ganglion is fairly distinct in lateral view, and more so in latero-ventral view (Fig. 57, ggl. ceph. 1). It is situated between the oesophagus and the lateral band, and is composed of about 15 cells, similar in shape to those of the ventral ganglion. The bunch of cells measures about 42μ in length, and is thicker on the anterior part where it connects with the nerve ring. Each cell appears connected by a thin fibre with the nerve ring so that the cells are not all massed together. In cross section, just posteriorly to the nerve ring, this ganglion is represented by 3-4 cells of about 8μ in diameter, situated between the oesophagus and the pulp of the lateral bands. The further connections of this ganglion with the rest of the central nerves will be referred to later.

Subcutaneous cephalic commissure.—If a living or recently dead *Haemonchus contortus* is examined on the ventro-lateral side under high magnification, it is possible to find just at the level of the nerve ring a fine strip, apparently composed of a few nervous fibres, running between the cuticle and subcuticle from the ventral to the lateral subcuticular bands in a direction perpendicular to the axis of the worm. In specimens dead for some time or in specimens cleared by glycerine I was not able to find this structure. No further investigations were made, but it appears that this strand of nerve fibres represents the "Commissura (ventro-lateralis) cephalica cutanea" described by Looss in the *Ankylostoma duodenale*. In living specimens, two other fine bundles of fibres are seen which have a common origin with the subcutaneous cephalic commissure. From the point of connection both bundles curve outwardly, joining the ventral sublateral bands.

The Cephalic Internal Commissure.—In ventro-lateral view numerous nerve anastomoses are seen between the cells of the cephalic ventral and the cephalic lateral ganglia (Fig. 57, nrv. anas.). The posterior anastomosis is a more conspicuous one and takes its origin in one of the posterior cells of the cephalic ventral ganglion. For a short distance it has a common course with the ventro-post-lateral commissure (*vide infra*), then turning

rather sharply anteriorly joins the posterior cell of the cephalic lateral ganglion (Fig. 57, co. ceph. vtr. lat. 2). This anastomosis closely corresponds with the cephalic internal commissure of *Ankylostoma duodenale*.

The Peripheral Nervous System.

Nerves of the Cephalic Papillae.—In specimens cleared in glycerine, and examined chiefly in ventral and dorsal view, the above nerves can easily be distinguished at their origin from the central nervous system. The two sub-median, dorsal, or ventral ones start directly from the nerve ring. In figure 57 are seen the two ventral nerves (nrv. pap. v.) and the dorsal one (nrv. pap. d.). The nerves of the lateral papillae commence from the lateral cephalic ganglion (Fig. 57, nrv., pap. l.). Each nerve is rather thin, and along the first portion a few small spindle shaped cells can be seen. On section each nerve appears composed of a few fibres running along the periphery of the oesophagus, the lateral ones between the oesophagus and the lateral bands, and the sub-median ones about midway in each oesophageal quadrant. These nerves are surrounded by a granular substance. In cross section closer to the mouth the above nerves cannot always be detected with certainty, but in a whole specimen it is sometimes possible to trace their course until they reach the cephalic papillae.

The Post-lateral Cephalic Ganglia.—From the posterior end of the cephalic lateral ganglion a few nerve fibres (which were described by Looss in the *Ankylostoma duodenale* as fibres of the lateral cephalic papillae) run on the inner surface of the lateral bands until they reach the base of the cervical papillae, or a point slightly beyond it. Here they join two or three ganglion cells, situated on the dorsal part of the lateral bands. These cells represent the post-lateral cephalic ganglion (Fig. 57, ggl. ceph. post-lat.).

Nerves of the Cervical Papillae.—These nerve fibres leave the post-lateral ganglion through the pulp of the lateral band, usually making a slight curve anteriorly and entering the base of the cervical papillae (Fig. 57, nrv. pap. cerv.).

Ventro Post-lateral Commissure.—This is rather a conspicuous nerve, having its origin together with the cephalic internal commissure at the ventral cephalic ganglion. It then runs posteriorly and laterally, joining the post-lateral cephalic ganglion. (Fig. 57, co. ceph. ventr. post-lat.).

Longitudinal Nerves.

Ventral Nerves.—The ventral nerve starts from the ventral cephalic ganglion, and is composed of two bundles of nerve fibres. Each bundle consists of nerve fibres starting directly from the more superficial layer of the nerve ring and of others starting from the cells of the cephalic ventral ganglion (Fig. 57, nrv. vtr.). The two resulting branches of the ventral nerve are thus seen gradually converging towards the median line, until they join together at the level of the anterior end of the cervical glands. From this point the trunk of the ventral nerve proceeds backwards on the inner surface of the ventral band. In the female, just in front of the

vulva, the ventral nerve enters a small ganglion, called the "vulval ganglion." From this ganglion a thin nerve fibre goes into the vulval linguiform process. The trunk of the ventral nerve appears posteriorly to the above ganglion, having, after bifurcation, encircled the vulva. Proceeding backwards, it again appears in a single trunk. Frequently, ganglion cells are present along its course, from which lateral nerve fibres take their origin.

Lateral Nerves.—From the posterior end of the lateral cephalic ganglion another large bundle of fibres takes origin. These soon reach the ventral margin of the lateral band, where they receive a few fibres from the post-lateral cephalic ganglion and run backwards along the lateral band. They represent the "ventro lateral nerves."

Sub-lateral Nerves.—The origin of the ventral sub-lateral nerves has been described in dealing with the "subcutaneous cephalic commissure." There is ground to admit the presence of the dorso sub-lateral nerves in view of the fairly well marked development of the sub-lateral bands. Along the length of the body transverse nerve fibres run across the lateral bands, or run from one side to the corresponding sub-lateral bands.

Dorsal Nerve.—In latero-dorsal view of cleared specimens a very thin bundle of fibres is seen to start from the posterior margin of the nerve at the level of the dorsal band. These nerve fibres join the inner surface of the dorsal band after describing a slightly oblique course where they enter two ganglion cells. These cells represent the "dorsal ganglion." From the dorsal ganglion the dorsal nerve, which is very thin, runs along the inner surface of the dorsal band throughout its length.

Nerves of the Posterior end in the Female.

Ganglia Anal.—At the posterior end of the chyle intestine the ventral nerve (Figs. 58, 59, nrv. vtr.) is divided into two bundles of fibres, each one passing through a longitudinal group of ganglion cells, which correspond to the "Anal ganglia" (Figs. 58, 59, ggl. an.). These two ganglia diverge slightly posteriorly, and the cells are scattered and not very conspicuous. Posteriorly to the ganglion the bifurcations of the ventral nerve turn laterally and anteriorly, each describing an arc which joins the lumbar ganglia. These arcs represent the "ano-lumbar commissure" (Figs. 58, 59, co. ano. lumb.).

Lumbar Ganglia.—In examining a clear specimen from the ventral side, two groups of nerve cells are seen, each adhering to the lateral bands on each side of the ano-rectal ligament. They represent the "lumbar ganglia." Each ganglion is about 64μ long and consists of two pairs of cells, with an unpaired cell at both ends (Figs. 58, 59, ggl. lumb.). The ventro-lateral nerve enters this ganglion and then proceeds backwards. At the level of the anus two or three oval-shaped nerve cells are still seen enclosed in the same lateral nerve. The nerve ends at the caudal papillae (Figs. 58, 59, nrv. lat. v.). It may be mentioned here, that in the same ventral view of the worm, just behind the end of the genital tube, a rather conspicuous bundle of nerve fibres can be seen situated obliquely to the axis of the body and apparently running from the right ventral band to

the left. These fibres then run posteriorly for a short distance and enter into five or six large ganglion cells. From these cells a similar bundle of fibres is seen running towards the median line of the body. I am not in the position to say what they represent.

Rectal Ganglion.—The rectal ganglion is also well represented in *Haemonchus contortus* by six or seven conspicuous cells situated under the anterior nose-like protuberance of the pulvillum post analis, and consequently lying dorsally to the rectum (Figs. 58, 59, ggl. rect.). The cells are rather close together and form a well defined group. From both lateral sides of the anal ganglion a bundle of nerve filaments is sent to the rectal ganglion. This bundle of fibres represents the “ano-rectal commissure” (Figs. 58, 59, co. ano. rect.).

The Anal Ring.—From the anal ganglion and between the origin of the ano-rectal and ano-lumbar commissure a bundle of two or three nerve fibres starts independently, directed dorsally around the rectum and having the appearance of a well defined ring. Looss does not describe this ring in *Ankylostoma duodenale*, but it apparently corresponds to the structure in *Ascaris*, called the “Anal Ring” (Figs. 58, 59, an. ri.). Sometimes the nerve fibres of the anal ring are very numerous, forming a broad band. A few thin nerve fibres run along the dorsal wall of the rectum, connecting the anal ring with the rectal ganglion.

Dorsal Nerve.—In the rectal region the dorsal nerve is represented by a thin bundle of nerve fibres, which at the level of the anal muscle appears turned ventrally, joining a conspicuous nerve ganglion. This ganglion is situated dorsally to the anus and rather close to it. (Figs. 58, 59, ggl. nrv. d.).

Nerves of the Posterior End in the Male.

Anal Ganglia.—As in the female the ventral nerve is broad in its posterior portion. At the level of the anterior part of the rectum it bifurcates and on each side enters two ganglia situated on the ventral bands. The more anterior one is called the “Anal Ganglion” (Fig. 60, ggl. an.), and the posterior one the “sub-Anal Ganglion” (Fig. 60, ggl. suban.). These two ganglia can easily be confused with neighbouring structures and their presence is not always detected. Proceeding from each sub-anal ganglion is a thin nerve lying along the ventral wall of the cloaca, corresponding to the “Terminal Nerve” (Fig. 60, n.v. term.).

Lateral Ganglia.—At the level of the base of the spicule the ventro-lateral nerve is seen lying along the ventral side of the lateral band. In its posterior course three successive ganglia are found. The more anterior is called the “lumbar ganglion” and is found at the level of the anterior end of the gubernaculum (Fig. 60, ggl. lumb.). Viewed dorsally it is covered by the musculus sedator gubernaculi. It is composed of two or three cells, the more dorsal one being the largest. This cell is oval shaped, measures about 28μ in its widest part, and contains a distinct nucleus. By the side of the ano-lumbar commissure (*vide infra*), two other strong nerves take origin from the lumbar ganglion, and are directed posteriorly and medially. The next is the “post-lumbar ganglion.” It

is situated at about the level of the pre-bursal papilla, and consists of six or seven oval cells adhering to the lateral bands (Fig. 60, ggl. post-lumb.). The more posterior ganglion is called the "Costal ganglion." It is seen on the base of the main trunk of the bursa and sends nerves to the lateral and dorsal region (Fig. 60, ggl. cost.).

The Ano-Lumbar Commissures.—The anal ganglia are connected with the lumbar ganglia by two commissures. The more anterior is the "Ano-Lumbar Commissure," connecting each anal ganglion with the lumbar ganglion of the same side (Fig. 60, co. ano. lumb.). It is rather short and describes in its course a slight posterior convexity. The second one is the "Sub-Ano Post Lumbar Commissure," connecting the sub-anal ganglion with the respective post lumbar ganglion (Fig. 60, co. suban. post-lumb.). The posterior convexity described by these commissures is quite distinct, and about midway a nerve starts posteriorly directed to the ventral rays of the bursa (Fig. 60, nrv. v. ray.).

Rectal Ganglion.—In the dorsal view of a cleared specimen the rectal ganglion is rather conspicuous. It is situated slightly anteriorly to the gubernaculum above the spicular canal, and has a typical horse-shoe formation (Fig. 60, ggl. ret.). It appears to be composed of four ganglion cells pressed rather close together. It is connected on each side by a conspicuous bundle of fibres proceeding to the pair of anal ganglia. These fibres represent the "ano-rectal commissure" (Fig. 60, co. ano. ret.). Sometimes I found the ano-rectal commissure directed posteriorly (Fig. 60) from the anal ganglion instead of anteriorly. This fact is explained by displacement of the spicular apparatus.

Sub-Rectal Ganglion.—This ganglion was not very distinct, but two bundles of thin nerve fibres were seen starting from each side of the anal ganglion (Fig. 60, co. ano. sub. ret.). These fibres correspond very closely to the similar structure identified by Looss as the "Ano Sub-Rectal Commissure" of the *Ankylostoma duodenale*.

Excretory Apparatus.

The excretory pore is found in the ventral side of the body, situated between the two divergent parts of the cephalic ventral ganglion (Fig. 57, p. ex.). On the cuticle it appears like a small opening surrounded by a round, flat area, where the anular striations of the skin are missing. The excretory vesicle can hardly be seen in fixed specimens, even in those cleared in glycerine, and it appears always deformed (Fig. 57). In living worms, or worms freshly killed, examined in water between a slide and a cover glass, a pear-shaped vesicle about 250μ long appears quite clearly on lateral view, and is connected by the thin anterior end with the excretory pore, running nearly parallel to the walls of the body between the ventral band and the oesophagus. At the posterior end it receives the two efferent ducts of the cervical glands. The degree of expansion of this vesicle varies in different specimens and sometimes it is found contracted to such an extent that it appears as a thin line.

Bridge of the Excretory Apparatus.—In glycerinated specimens observed in ventral view a spindle-shaped deviation from the ventral side of the

lateral band is seen just anteriorly to the nerve ring. In fresh specimens observed in lateral view it is also possible to distinguish a strand of fibres connecting the above spindle-shaped body and reaching the ventral band at the level of the excretory pore. It appears that the spindle-shaped body and the fibre-like strand represent the "suspensory cell of the excretory apparatus" described in the *Ankylostoma duodenale* by Looss. In addition the "carrying cell of the excretory vesicle," and the "carrying cell of the cervical glands" described by the same author, appear to be represented by a granular mass surrounding the excretory vesicle.

The body of the cervical glands starts anteriorly at the level of the angle formed by the cervical papillae and the body walls. The anterior end is smooth and round (Fig. 57, gl. cerv.). The first portion of the cervical glands is rather thick, slightly compressed dorso-ventrally and occupying nearly all the space between the œsophagus and the ventral walls of the body (Fig. 45, gl. cerv.). At the level of the thicker part of the œsophagus the glands are reduced in diameter by the pressure of that organ. Proceeding posteriorly the diameter of the glands lessens until about midway. The posterior half of the gland gradually increases in diameter and then decreases, assuming roughly a lancet shape. The right gland is usually slightly thicker than the left one. The nucleus lies at the level of the largest diameter of the posterior portion. The total length of the gland varies from 3.7-4 mm. In the female the cervical glands are frequently seen to be slightly shorter than they are in the male. The right gland exceeds the length of the left one by a few hundred microns. Throughout their length the cervical glands lie free in the coelomic cavity, kept in a latero-ventral position by the pressure of the intestine, and separated from each other. The pointed posterior tip of the gland is adherent to the ventral walls of the intestine. This fact can be clearly seen on cutting a fresh worm just posteriorly to the œsophagus and placing the specimen on a slide with some drops of water. As soon as the two pieces of the body are separated the chyle intestine in the posterior portion of the specimen protrudes for some distance from the coelomic cavity. The posterior portions of the cut cervical glands are then seen to lie free in the water except for the adherence of the posterior tip.

Peripheral Excretory Canals.—These two canals wind along the ventral portion of the lateral band. The anterior portion of each canal starts from the head and ends in the excretory vesicle at the level of the excretory bridge (Figs. 43-44, ex.). The posterior portion forms a common junction with the anterior portion before reaching the excretory vesicle, and ends posteriorly at the level of the anus in the female and at the base of the bursa in the male (Figs. 46-47, ex.). The connection between the excretory canals and the excretory vesicle is difficult to detect. Fresh specimens are better for this purpose than those cleared in glycerine.

Cephalic Glands.—The excretory canal of the cephalic gland does not appear distinct in glycerinated specimens. In living or freshly killed worms, it appears as a thin clear canal opening through a small hole in the neighbourhood of the lateral cephalic papillae. The excretory canal is surrounded by a granular strand of the cephalic gland, increasing in thickness posteriorly, and compressing the substance of the lateral bands.

In the precerebral portion, the cephalic glands are bean-shaped in cross section. They measure about 9μ by 4μ , and are situated between the two bridges of the lateral bands and the œsophagus (Fig. 43, gl. ceph.). Just behind the nerve ring the cephalic glands compress the lateral bands. At this point they measure 16μ in diameter and are uniformly granular (Fig. 45, gl. ceph.). More posteriorly they are reduced considerably by the pressure of the œsophageal end. The nucleus of the cephalic gland is situated just behind the base of the cervical papilla. In the male the nucleus is about 28μ long, 16μ broad, and is somewhat darker than the surrounding tissue (Fig. 45, nu. gl. ceph.). Between the œsophagus and the genital tube, the cephalic gland increases in size, reaches 14μ in diameter and presses against the pulp of the lateral bands. Proceeding backwards the granular body of the cephalic gland steadily decreases in size. It was not found possible to detect any traces of the cephalic gland in cross sections made at, or beyond, the level of the seminal vesicle in the male or the initial ovarian spiral of the female.

PLATES.

- Fig. 1.— Egg of *Haemonchus contortus*, taken from the distal portion of the uterus of a young worm preserved in glycerine (380 diam.).
- Fig. 2-3.— Eggs of *Haemonchus contortus*, taken from the distal portion of the uterus of a well-grown worm preserved in glycerine (380 diam.).
- Fig. 4.— Egg of *Haemonchus contortus*, taken from the median portion of the uterus of a well-grown worm preserved in glycerine (380 diam.).
- Fig. 5.— Egg of *Haemonchus contortus*, taken from the proximal portion of the uterus of a well-grown worm preserved in glycerine (380 diam.).
- Fig. 6.— Egg of *Haemonchus contortus* (11-cell stage), from the stomach contents of an infected sheep (380 diam.). Note the four ectoderm cells of the right side disposed in the form of a cross.
- Fig. 7.— Egg of *Haemonchus contortus* (26-cell stage), from the stomach of an infected sheep (380 diam.). Note the eight ectoderm cells of the right side disposed in the form of a rosette.
- Fig. 8.— Egg of *Haemonchus contortus* at "morula stage," from fresh faeces of a sheep (380 diam.).
- Fig. 9.— Egg of *Haemonchus contortus* between the morula and [the tadpole stage. Dorsal view. From faeces of a sheep a few hours after having been passed. Ambient temperature 18° C. (380 diam.).
- Fig. 10-11.—Eggs of *Haemonchus contortus* at "tadpole stage." In Fig. 10 the embryo is seen in lateral view. In Fig. 11 the embryo appears more developed, and is seen in latero-ventral view. Both eggs from faeces of a sheep 10 hours after having been passed. Ambient temperature 18° C. (380 diam.).
- Fig. 12-13.—Eggs of *Haemonchus contortus* with the embryo about two or three times the length of the egg-shell. From faeces of a sheep fifteen hours after defaecation. Ambient temperature 18° C. (380 diam.).
- Fig. 14.— Egg of *Haemonchus contortus* with "mature embryo." From faeces of a sheep eighteen hours after having been passed. Ambient temperature 18° C. (380 diam.).
- Fig. 15.— Newly hatched larva of *Haemonchus contortus*. Killed with hot water (380 diam.).
- Fig. 16.— Larva in the first stage. External view, lateral aspect. The larva is magnified by 380, while the lateral line is seen with a magnification of 1400 diam.
- Fig. 17.— Larva in first lethargus (380 diam.).
- Fig. 18.— Larva in the first ecdysis, casting off the old skin. From a living worm in a culture with liquid medium. The tail of the old skin is hooked and attached to small solid particles at the bottom of the culture; the body of the larva is "whipping" in the liquid medium (380 diam.).
- Fig. 19.— Larva in the beginning of the second stage, lateral view, from specimen killed with hot water (380 diam.).
- Fig. 20.— Larva just before the second lethargus. From a living worm in a liquid medium. External appearance. Lateral view. The lateral line is slightly exaggerated in breadth, and is drawn as it was seen in a magnification of 700 diam. The larva is magnified by 380.
- Fig. 21.— Mature larva, after about a week of maturity. The larvae were grown in faeces, and attained a maximal length. The more external line represents the old skin (380 diam.).
- Fig. 21A.— Transversal section of a mature larva, made in the anterior portion of the chyle intestine. The section was made by the freezing process (1600 diam.).

- Fig. 22.— Mature larva two months old and still alive, collected on the walls of a jar, where the moisture was rather scarce. Note the signs of age, viz., contraction of the larva, decrease of granulations, and appearance of vacuoles in the chyle intestinal cells, disappearance of the internal structure (380 diam.).
- Fig. 23.— Mature larvae preserved in ice for three and a half months, and found dead. The outer skin is almost completely filled by the larva. The chyle intestinal cells are replaced by vacuoles, and the internal structure has disappeared (380 diam.).
- Fig. 23A.— Culture of larvae in a jar. The colony of mature larvae is seen on the walls in ascending migration.
- Fig. 24.— Anterior end of a larva in the parasitic part of the third stage (first parasitic stage), just before the third lethargus (1600 diam.).
- Fig. 25.— Posterior end of a larva in the parasitic part of the third stage (first parasitic stage), just before the third lethargus (1100 diam.).
- Fig. 26.— Larva casting the old skin at the completion of the third ecdysis (150 diam.).
- Fig. 27.— Anterior end of a larva in the beginning of the fourth stage (second parasitic stage). The thickness of the head cuticle is somewhat exaggerated (1600 diam.).
- Fig. 28.— Posterior end of a larva at the beginning of the fourth stage (second parasitic stage) (1400 diam.).
- Fig. 29-30.—Posterior end of the male and female in the fourth stage, three days after infection of the host. To illustrate the first structural differences appearing in the distinction of the two sexes.
 Fig. 29.—Male (180 diam.).
 Fig. 30.—Female (95 diam.).
- Fig. 31-32.—Posterior end of male and female in the fourth stage, 4-5 days after infection of the host. To illustrate the development of the genital rudiment and the difference in the tail.
 Fig. 31.—Male. The posterior end does not yet appear bilobated. The lateral lobes of the bursa are not yet defined (180 diam.).
 Fig. 32.—Female (100 diam.).
- Fig. 33-34.—Posterior end of male and female in the fourth stage, 6-7 days after infection of the host.
 Fig. 33.—Male. Posterior end of the body bilobated. Outlines of the bursa distinct. Genital tube reaches the cloaca (100 diam.).
 Fig. 34.—Female (180 diam.).
- Fig. 35.— Anterior end of female just before the fourth lethargus. Nine days after infection of the host (1040 diam.).
- Fig. 36.— Nine to ten days after infection of the host. Anterior end of female in the fourth ecdysis. The old skin is separated from the body (1040 diam.).
- Fig. 37.— Portion of the body of a female in the region of the vulva (fourth ecdysis). The old skin is separated from the body (140 diam.).
- Fig. 38.— Posterior end of a female in fourth ecdysis (300 diam.).
- Fig. 39.— Posterior end of a male in fourth ecdysis. Nine to ten days after infection of the host. The old skin is already detached, but not separated from the body (560 diam.).
- Fig. 40.— Adult male preserved in glycerine. From the ventral side (12 diam.).
- Fig. 41.— Adult female preserved in glycerine. From the left side (12 diam.).
- Fig. 41A.— Portion of a ventral muscular quadrant showing the connection of the muscular cells of two neighbouring rings (260 diam.).
- Fig. 42.— Longitudinal section of the head of an adult male preserved in glycerine. The section is made through the median line of the dorsal lip and the ventral cephalic papilla of the right subventral lip (1700 diam.).
- Fig. 43.— Section through the body of a male at the anterior end of the œsophagus (600 diam.).
- Fig. 44.— Section through the body of a male at the anterior edge of the nerve ring. The cell of the dorsal ganglion was drawn from a posterior section (600 diam.).

- Fig. 45.— Section through the body of a male at the base of the cervical papillae (600 diam.).
- Fig. 46.— Section through the body of a male at the level of the seminal vesicle (250 diam.).
- Fig. 47.— Section through the body of a male at the level of the anterior portion of the gubernaculum (250 diam.).
- Fig. 48.— Left ventral lobe of the bursa seen from the internal surface (130 diam.).
- Fig. 49.— Section through the left lateral lobe of the bursa at the level of the origin of the asymmetrical dorsal lobe (250 diam.).
- Fig. 50.— Section through the left lateral lobe of the bursa at the level of the tip of the externo-dorsal ray (250 diam.).
- Fig. 51.— Section through the body of a female in the last portion of the anterior ovary (180 diam.).
- Fig. 52.— Genital tubes in a female in the region of the vulva. The anterior uterus and ovijector, the unpaired tube, and the vagina show the internal structure. The posterior ovijector and uterus show the external muscular coat. The two punctated lines mark the course of the intestine (90 diam.).
- Fig. 53.— Section through the body of a female, midway through the anterior uterus (180 diam.).
- Fig. 54.— Section of the anterior portion of the "pars haustrix" (180 diam.).
- Fig. 55.— Section of the posterior portion of the "pars ejectrix" (180 diam.).
- Fig. 56.— Section of the female at the base of the linguiform process of the vulva (180 diam.).
- Fig. 57.— Nervous system in the anterior end of the body. Left latero-ventral view. From a female 18 days old and 17 mm. in length (320 diam.).
 (In calculating the distance between the different structural details the perspective should be taken into consideration).
- Fig. 58.— Nervous system in the posterior end of the body of a female, lateral view. From a specimen of about the same age and length as used in Fig. 57 (320 diam.).
- Fig. 59.— Nervous system in the posterior end of the body of a female, ventral view. From a specimen similar to that used for Fig. 57 (320 diam.).
- Fig. 60.— Nervous system of the posterior end of the body of a male, dorsal view. From a specimen as used in Fig. 57.

NOTE.—All the plates were drawn by the Abbe apparatus with Bernard table, and the magnification in diameters calculated by the same apparatus.

CHARTS.

- Charts No. 1-2.— Showing the effect of low temperature on the migration of larvae (see Thigmotropism of larvae—Temperature).
- Chart No. 3.— Showing the effect of changes in diffused light on the migration of larvae (see Phototropism of mature larvae—Changes in diffused light).
- Chart No. 4. — Showing the effect of cloudy weather on the migration of larvae (see Phototropism of mature larvae—Changes in diffused light).
- Charts No. 5-18.— Showing the migration of a colony of mature larvae under the alternating effect of day and night, and the final passage in the ground.

NOTE.—The Charts Nos. 1-18 are reduced to one-fourth from the original drawings.

————— = Level of the culture of faeces.
 — — — — — = Curve of the colony between sunset and sunrise.
 - - - - - = Curve of the colony in the day time.
 | | | | | | | | = Scale in centimetres.

REFERENCE LETTERS.

an. = anus.

an. ri. = anal ring.

ant. ov. = anterior ovary.

ant. nt. = anterior nterus.

b. dors. = dorsal band.

b. lat. = lateral band.

b. sub. = sublateral band.

b. vent. = ventral band.

buc. lan. = buccal lancet.

burs. = bursa.

burs. d. lob. = dorsal lobe of the bursa.

burs. l. lob. = lateral lobe of the bursa.

c. adv. l. = adventitious cells fixing the nerve ring to the lateral bands.

c. adv. v. = similar cells connecting the nerve ring with the ventral band.

c. l. lig. ret. = cells of the anterior ring of the rectal ligament.

c. v. ex. = cell containing the vesicle of the excretory system.

can. spic. = spicular canal.

chit. = longitudinal chitinous rod of the skin.

chit. = chitine.

chit. arc. 1. = anterior curvature of the walls of the mouth.

chit. arc. 2. = posterior curvature of the walls of the mouth.

chit. br. = bridge between the walls of the mouth and the walls of the body.

clo = cloaca.

co. ceph. = cephalic nerve commissure (nerve ring).

co. ceph. vtr. lat. 2. = internal cephalic commissure.

co. ceph. vtr. postlat. = ventro post-lateral commissure.

co. suban. = subanal commissure.

coel. = coelomarian muscular cell of the somatic muscular coat.

cut. ext. = external cuticle.

cut. int. = internal cuticle.

drs. lob. = dorsal lobe of the bursa.

drs. r. = ray of the dorsal lobe of the bursa.

drs. mar. = dorsal margin of the lateral lobes of the bursa.

ex. = excretory canal.

ex. drs. r. = externo-dorsal ray.

ex. gl. cerv. = excretory ducts of the cervical glands.

ex. l. r. = externo-lateral ray.

f br. marg. = marginal fibres of the oesophagus.

ge. co. = genital cone.

ggl. an. = anal ganglion.

ggl. ceph. l. = lateral cephalic ganglion.

ggl. ceph. postlat. = post-lateral cephalic ganglion.

ggl. ceph. v. = ventral cephalic ganglion.

ggl. cost. = costal ganglion.

ggl. dors. = dorsal cephalic ganglion.

ggl. lumb. = lumbar ganglion.

ggl. nrv. d. = ganglion in the course of the dorsal nerve between the two portions of the anal muscle.

ggl. nrv. ry. = ganglion of the nerve of the ventral rays.

ggl. ret. = rectal ganglion.

ggl. sec. = secondary ganglion in the course of the ventro-lateral nerve.

ggl. suban. = subanal ganglion.

gl. cem. = cement gland.

gl. cem. 1. = anterior portion of the cement gland.

gl. cem. 2. = posterior portion of the cement gland.

gl. ceph. = cephalic glands.

gl. cerv. = cervical glands.

gl. dors. l. = lateral stem of the dorsal oesophageal gland.
 gl. dors. m. = median stem of the dorsal oesophageal gland.
 gl. oes. subv. = subventral oesophageal glands.
 gn. pr. = genital primordium.
 gr. l. p. ejec. = external granular layer of the "pars ejectrix."
 gr. l. p. h. = external granular layer of the "pars haustrix."
 gr. tiss. = granular tissue.
 gran. cut. = deposit of granular masses within the internal layer of the skin.
 gub. = gubernaculum.

int. = intestine.
 int. gib. = internal gibbosity of the lateral lobes of the bursa.
 int. gr. = internal groove of the lateral lobes of the bursa.
 int. l. p. ejec. = internal lining of the "pars ejectrix."

l. lat. = lateral line.
 l. vulv. = lips of the vulva.
 lat. ves. = lateral vesicles of the linguiform process of the vulva.
 lig. rect. = rectal ligament.
 lin. proc. = linguiform process of the vulva.
 lt. ven. r. = latero-ventral ray.
 lu. oes. = oesophageal lumen.

m. an. = anal muscle.
 m. bas. burs. = basal muscle of the bursa.
 m. burs. = bursal muscles.
 m. cap. = mouth capsule.
 m. cav. = mouth cavity.
 m. ceph. oes. a. = anterior cephalo-oesophageal muscle.
 m. ceph. oes. p. = posterior cephalo-oesophageal muscle.
 m. cost. lat. ext. ant. = anterior externo-lateral costal muscle.
 m. cost. l. ext. post. = posterior externo-lateral costal muscle.
 m. cost. l. ext. post. r.i. = inner branch of the latter muscle.
 m. cost. l. int. = interno-lateral costal muscle.
 m. p. ejec. = musculature of the "pars ejectrix."
 m. p. h. = musculature of the "pars haustrix."
 m. sed. gub. = muscle "sedator gubernaculi."
 m. som. = somatic muscles.
 m. sup. gub. = supinator muscle of the gubernaculum.
 m. vag. = muscles of the vagina.
 ma. drs. } = dorsal and ventral margins of the lateral lobes of the bursa.
 ma. ven. }
 marg. or. = mouth opening.
 md. exs. spic. = exertor muscle of the spiculae.
 mu. oes. = oesophageal muscles.

nrv. anas. = nerves anastomosis between the lateral and ventral cephalic ganglia.
 nrv. co. = costal nerve.
 nrv. d. = dorsal nerve.
 nrv. gl. subv. = nerves of the sub-ventral oesophageal glands.
 nrv. lat. d. = dorso-lateral nerve.
 nrv. lat. = lateral nerve.
 nrv. pap. cerv. = nerves of the cervical papillae.
 nrv. pap. d. = nerves of the dorsal cephalic papillae.
 nrv. pap. l. = nerves of the lateral cephalic papillae.
 nrv. pap. v. = nerves of the ventral cephalic papillae.
 nrv. term. = terminal nerve.
 nrv. v. ray. = nerve of the ventral rays of the bursa.
 nrv. r. = nerve ring.
 nrv. vtr. = ventral nerve.
 nu. gl. ceph. = nucleus of the cephalic glands.
 nu. pars. h. = nucleus of an anterior cell of the "pars haustrix."

or. cav. = oral cavity.
 out. sk. = outer skin.
 ov. = ovary.
 ovij. = ovijector.

pap. caud. = caudal papilla.
 pap. cerv. = cervical papilla.
 pap. dors. = dorsal cephalic papilla.
 pap. lt. ven. r. = terminal papilla of the latero-ventral ray.
 pap. preab. = prebursal papilla.
 pap. v. = ventral cephalic papilla.
 pars. ejec. 1 = sphincter like portion of the "pars ejectrix."
 pars. ejec. 2 = cylindrical portion of the "pars ejectrix."
 pars. h.1 = one of the four anterior cells of the "pars haustrix."
 plat. = platymyarian muscular cell of the somatic muscular coat.
 proc. mu. = muscular processes.
 ps. l. r. = postero-lateral ray.
 pul. ray. = pulpa of the rays of the bursa.
 pulv. = pulvillum postanalis.

rect. = rectum.

rid. int. gr. = ridge of the internal groove of the bursa.

spic. = spicula.

sup. f. = superficial nerve fibres of the "commissura cephalica."

te. = testicular tube.

tru. l. lob. = common trunk of the rays of the lateral lobes of the bursa.

tru. ven. r. = common trunk of the ventral rays of the bursa.

u. part. = unpaired part of the genital tube of the female.

ut. = uterus.

v.s. = seminal vesicle.

vag. = vagina.

ven. mar. = ventral margin of the lateral lobes of the bursa.

ven. ven. ry. = ventro-ventral ray.

vulv. = vulva.

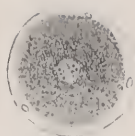
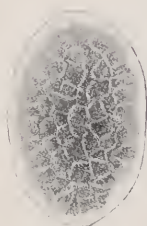
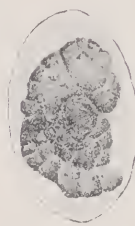
*Fig. I.**Fig. II.**Fig. III.**Fig. IV.**Fig. V.**Fig. VI.**Fig. VII.**Fig. VIII.**Fig. IX.**Fig. X.**Fig. XI.**Fig. XII.**Fig. XIII.**Fig. XIV.*



Fig. XV.



Fig. XVI.

Fig. XVII.



Fig. XVIII.



Fig. XX.



Fig. XIX.

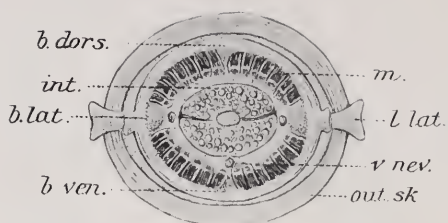
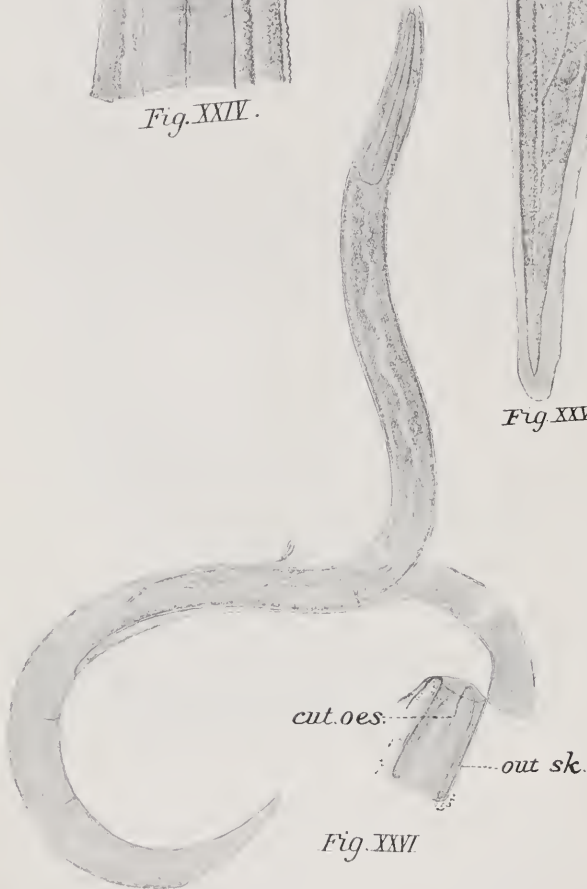
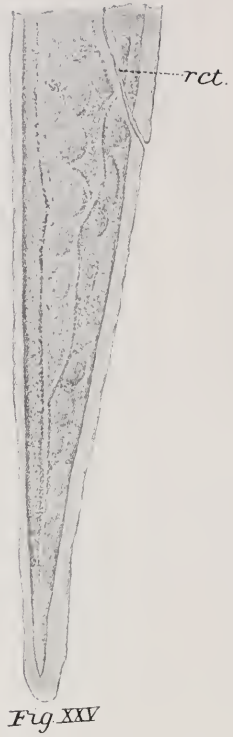
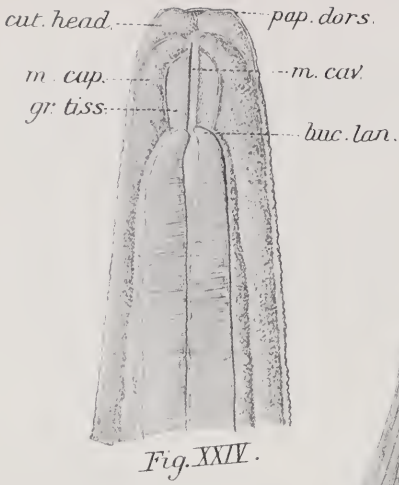
*Fig. XXI^a**Fig. XXI.**Fig. XXII.*



Fig. XXIIIa.

Fig. XXIII.

F. Veglia, del ad nat. (ex. Fig. XXIIIa).



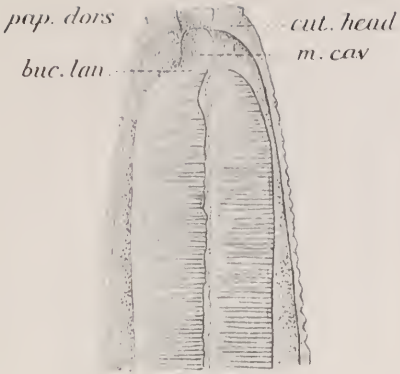


Fig. XXVII



Fig. XXVIII

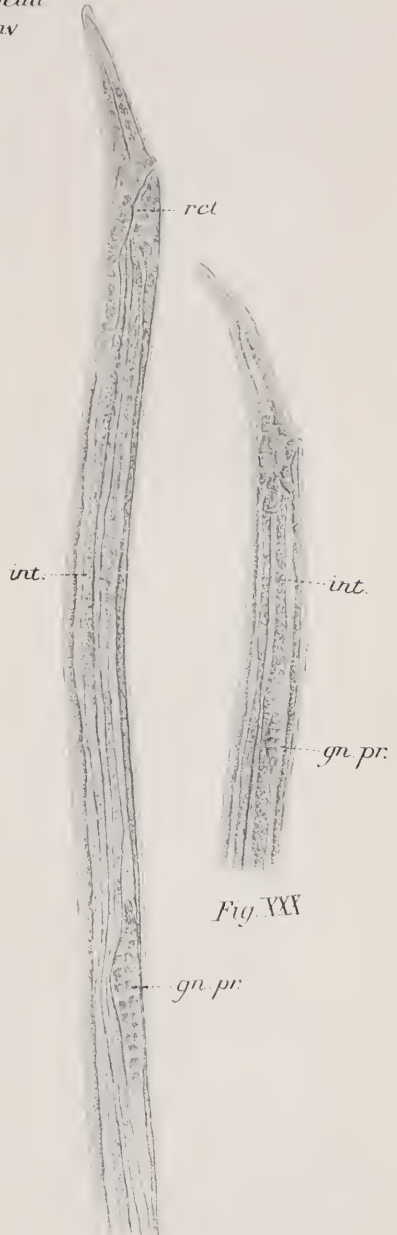


Fig. XXIX

Fig. XXIX

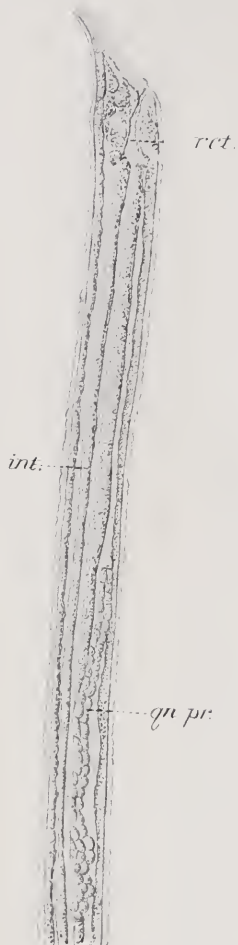


Fig. XXV



Fig. XXXII

F. Veglia, del. ad nat.



Fig. XXVIII.

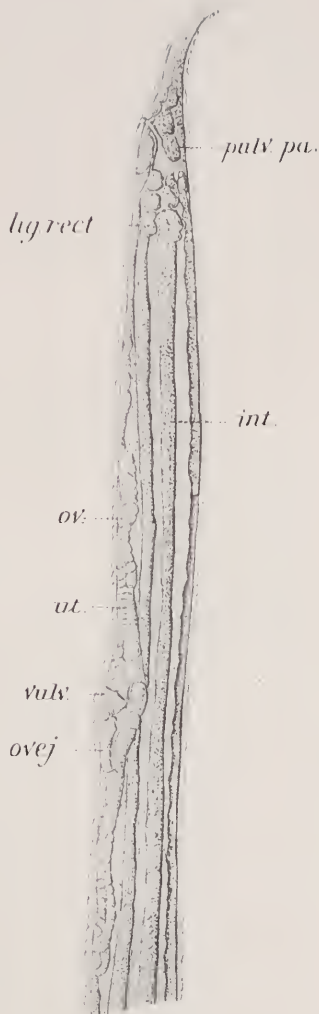


Fig. XXXIV

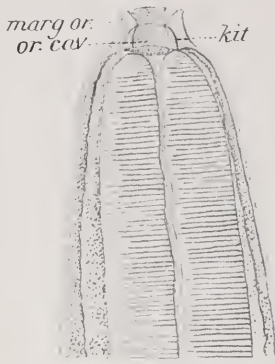


Fig. XXXV

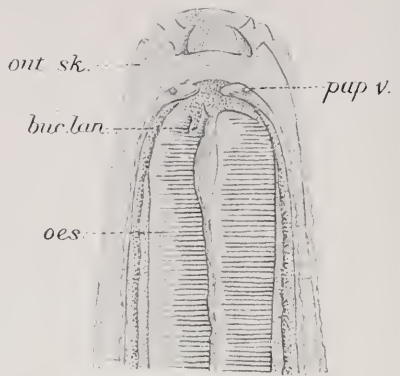


Fig. XXXVI

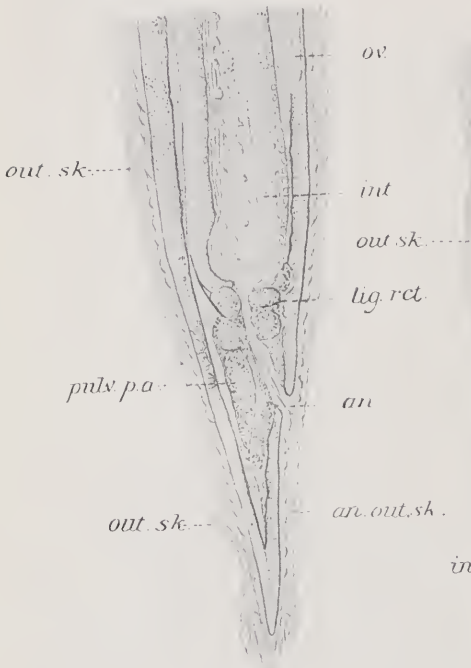


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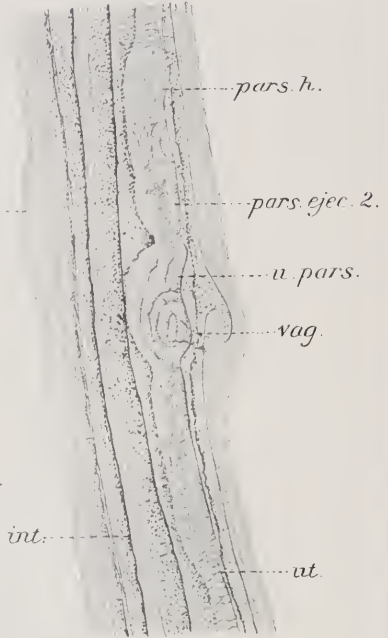


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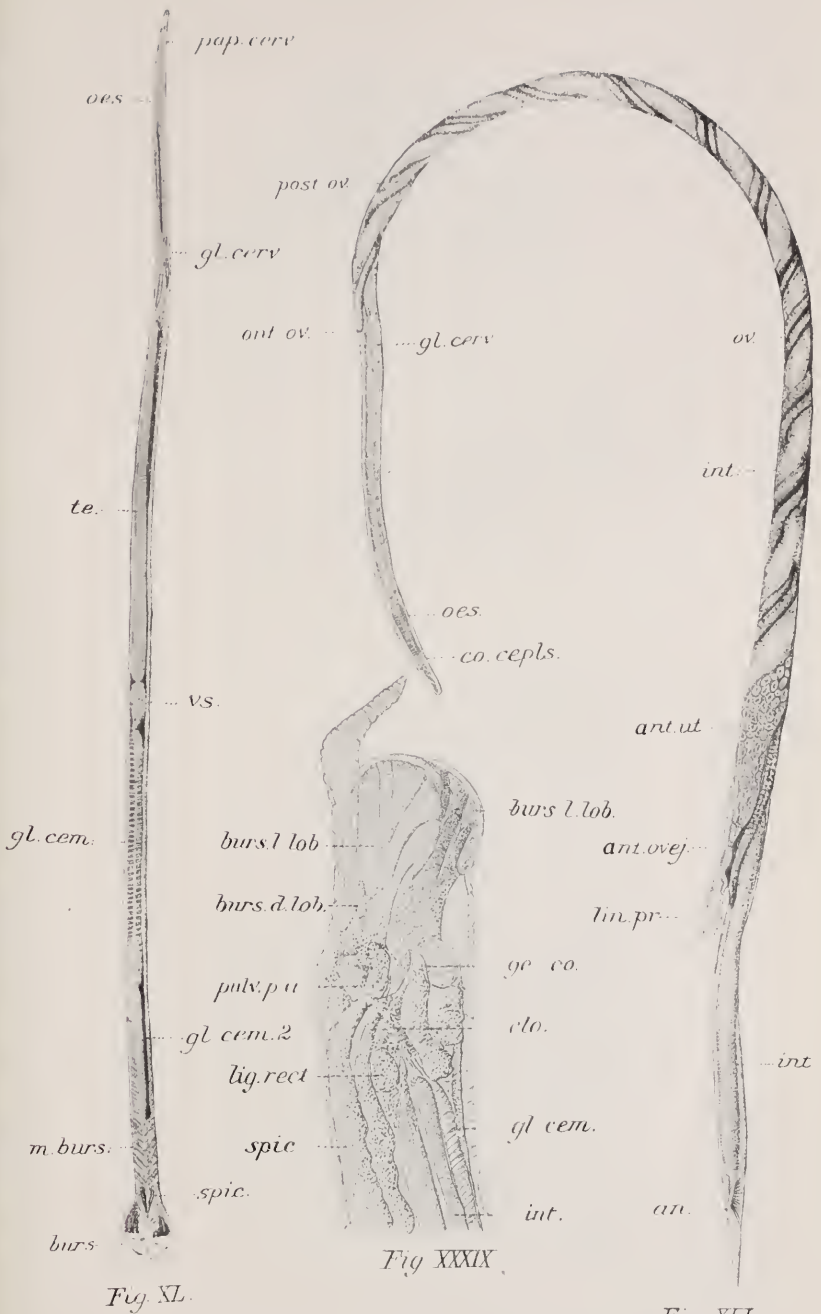


Fig. XXXIX.

Fig. XLII.

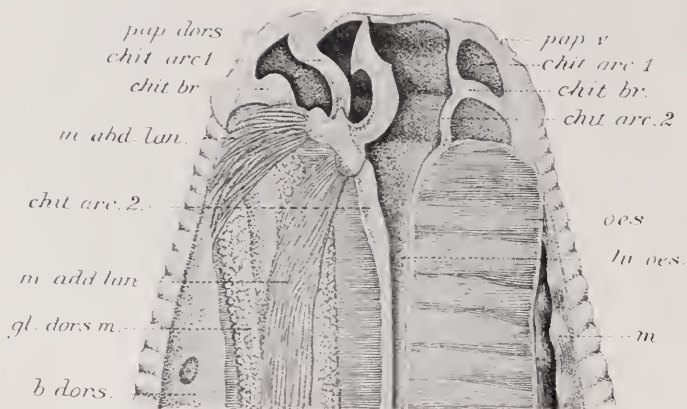


Fig. XLII.

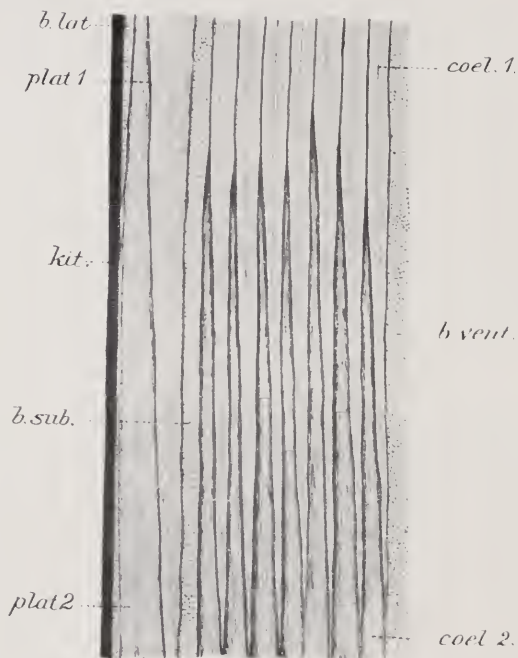


Fig. XLII.^a

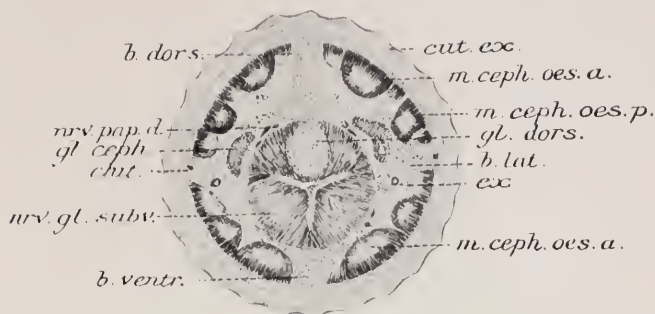


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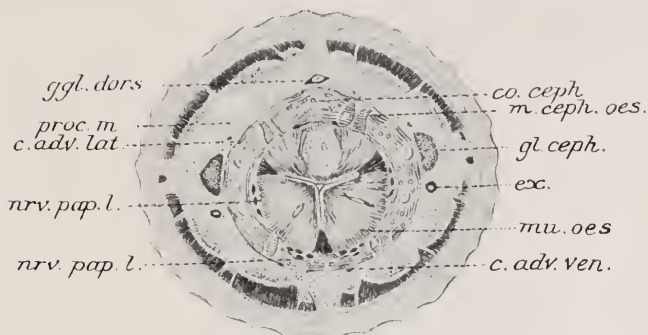


Fig. XLIV.

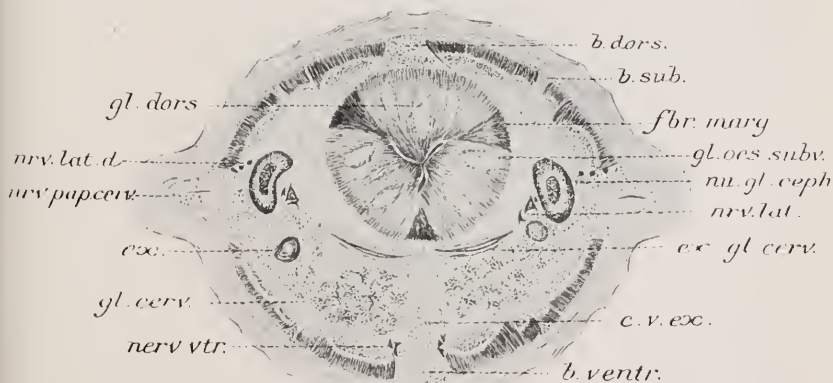


Fig. XLV.

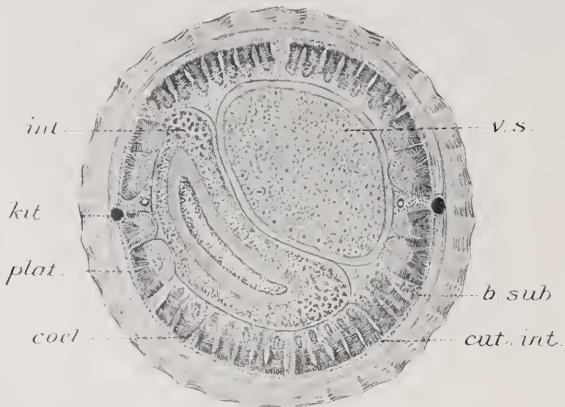


Fig. XLVI

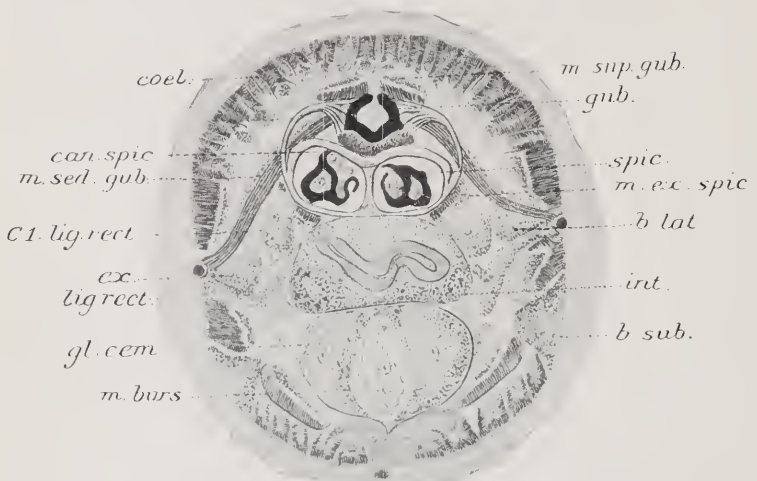


Fig. XLVII

F. Veglia, del. ad nat.

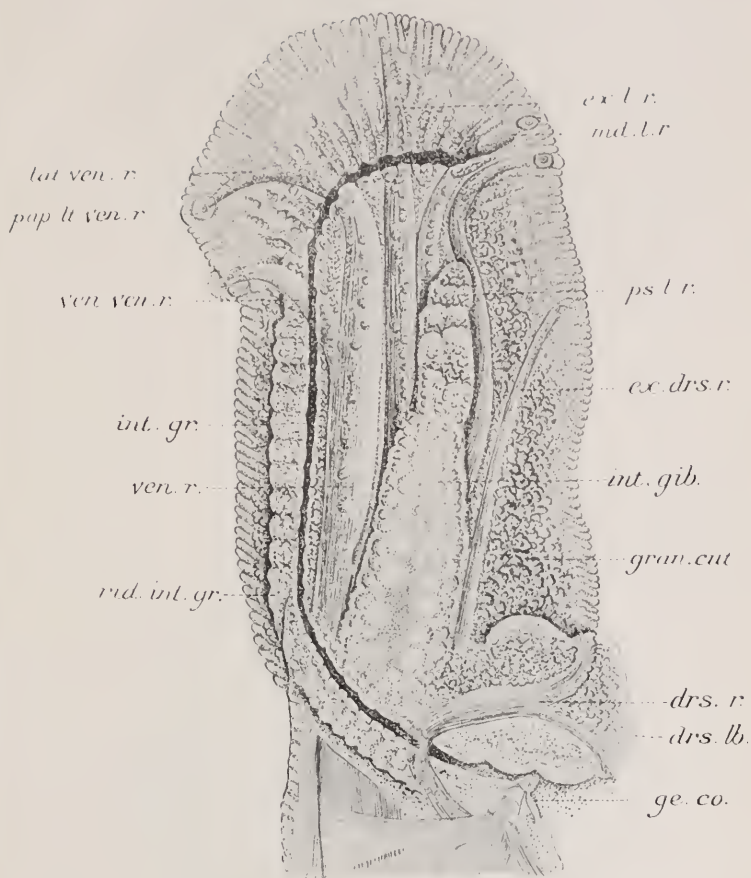


Fig. XLVIII.

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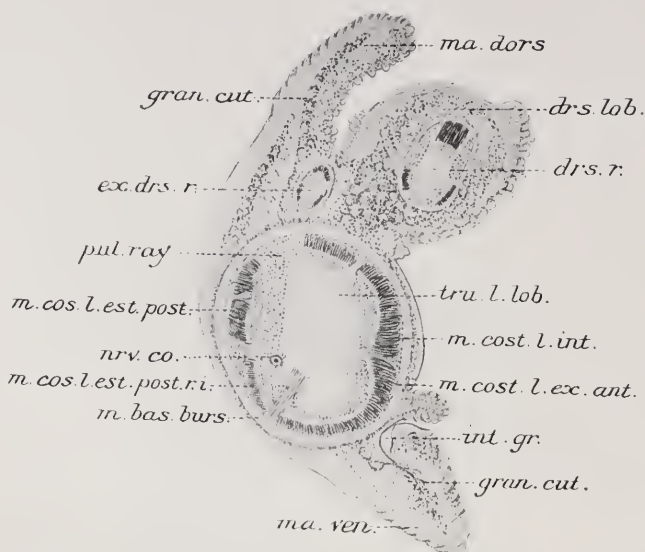


Fig. XLIX.

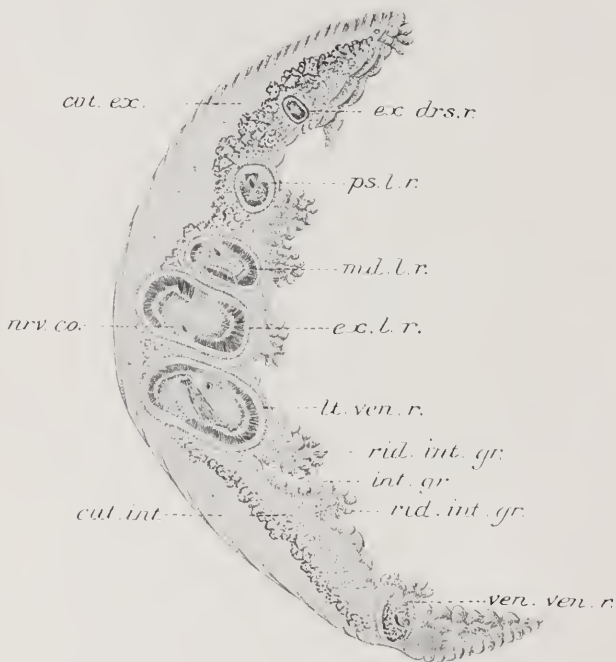


Fig. L.

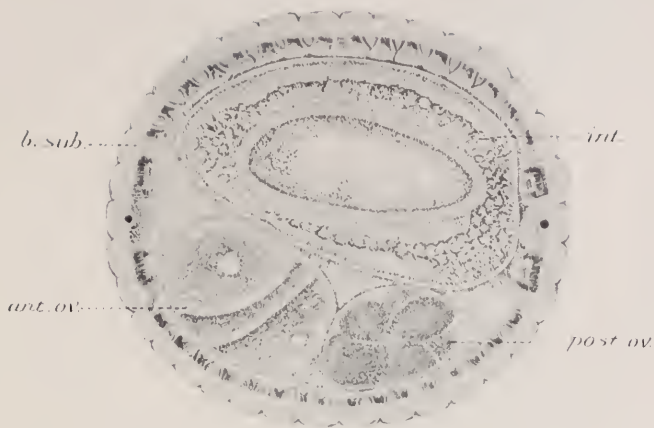


Fig. LI

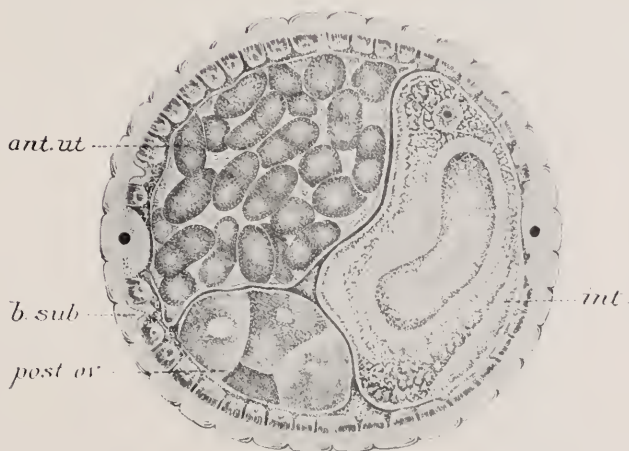


Fig. LIII.

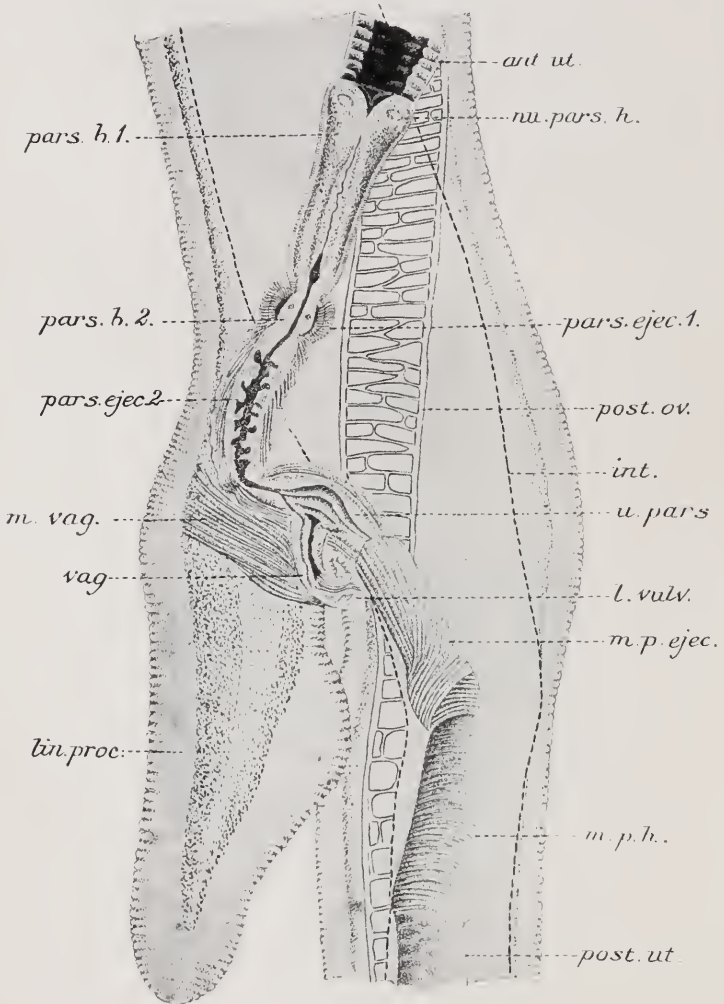
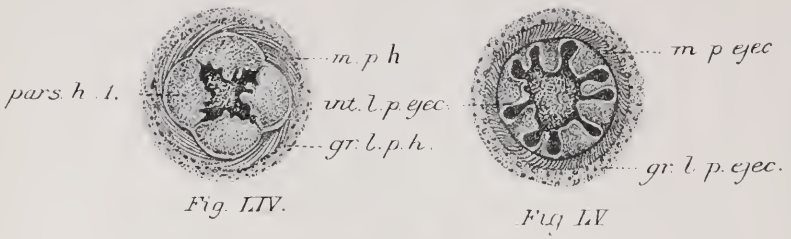


Fig. LVII.

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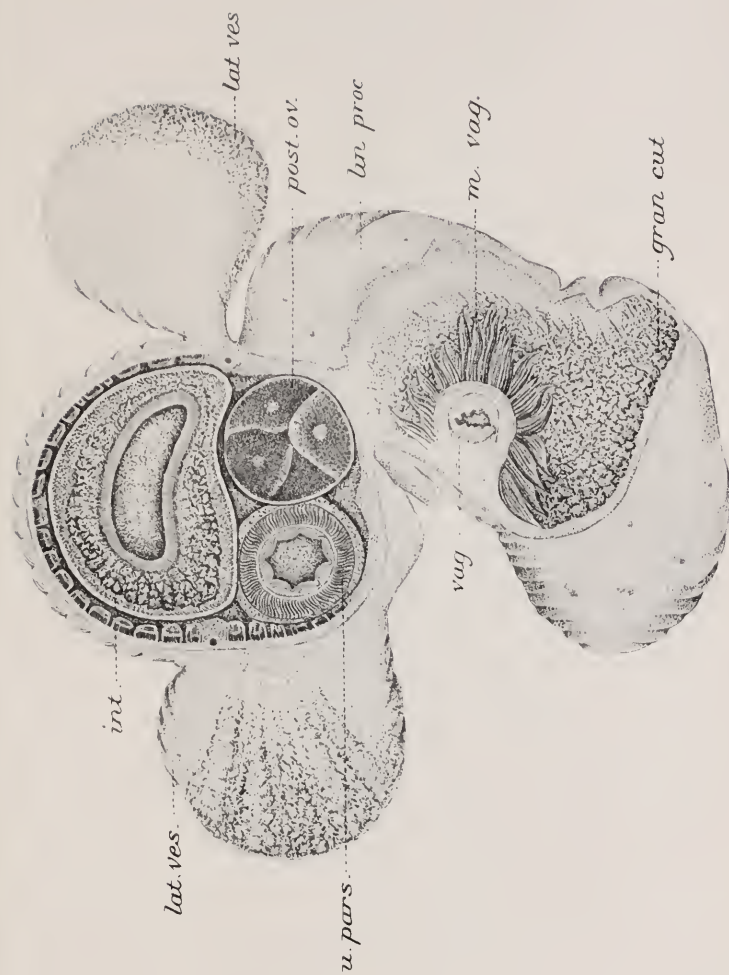


Fig. LVI.

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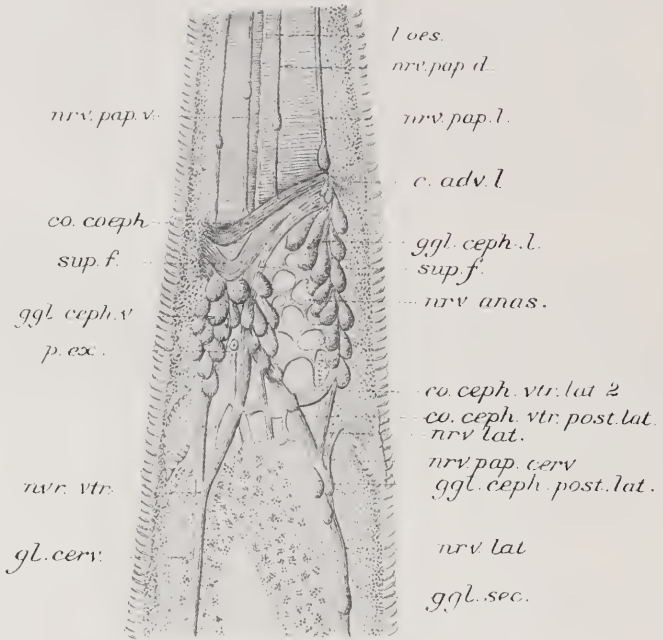


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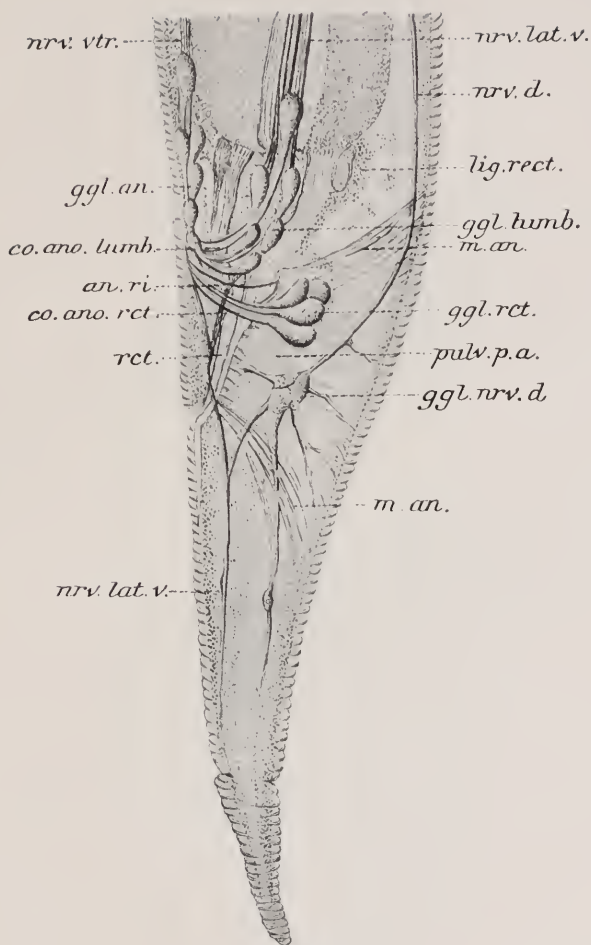


Fig. LVIII.

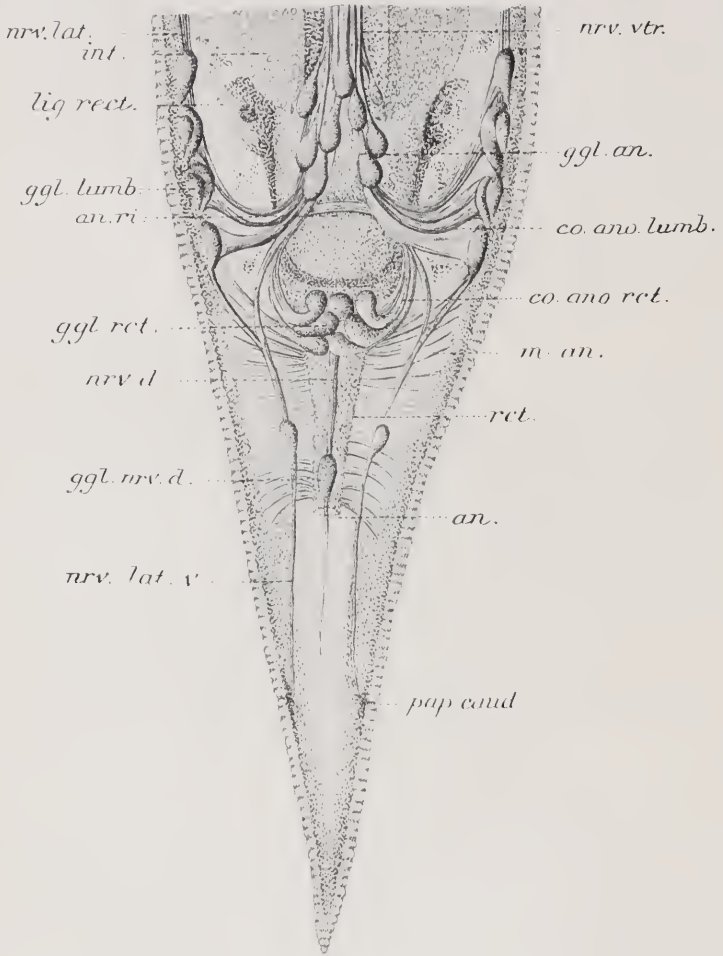


Fig LIX.

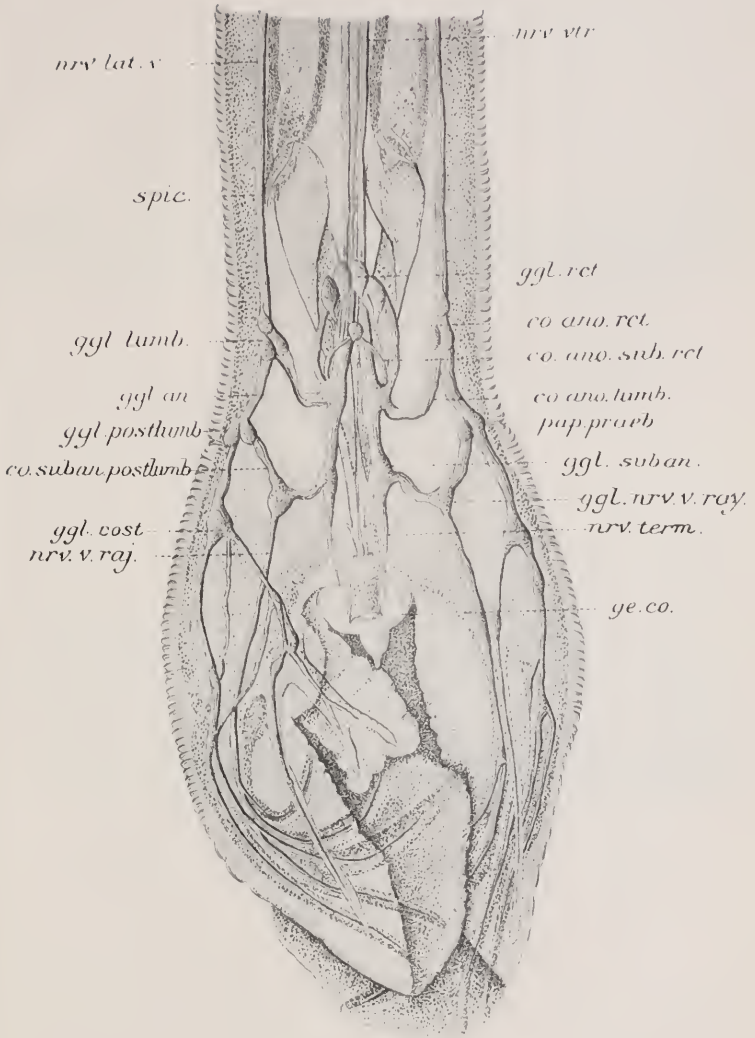


Fig. LX.



CHART 1.



CHART 2.



CHART 3.

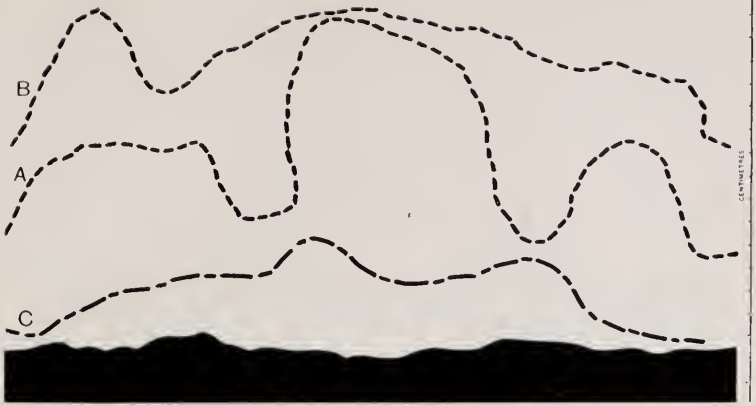


CHART 4.



CHART 5.

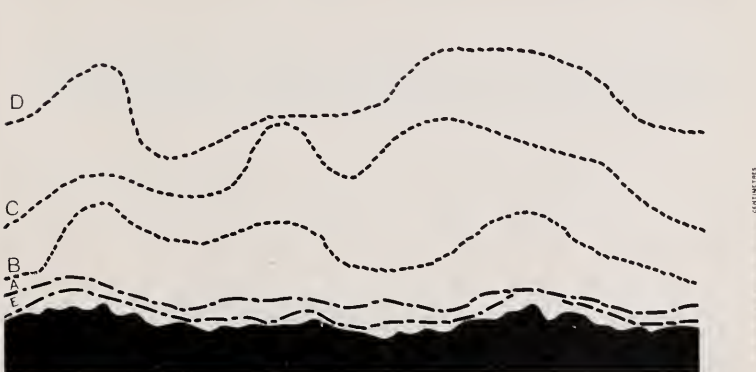


CHART 6.



CHART 7.

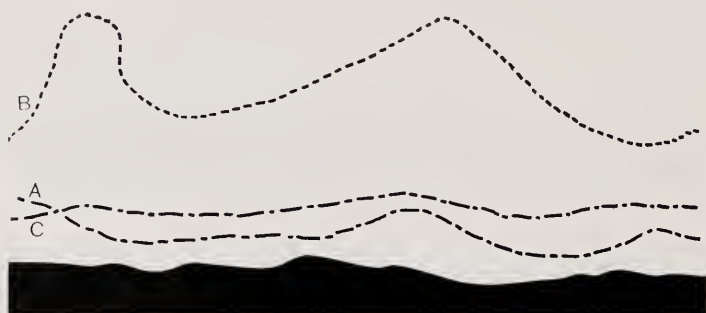


CHART 8.



CHART 9.



CHART 10.



CHART 11.



CHART 12.



CHART 13.



CHART 14.



CHART 15.



CHART 16.



CHART 17.



CHART 18.

**Some Observations in connection with the
Immunization of Cattle against South African
Redwater and Genuine Gallsickness
(Anaplasmosis).**

BY

JAMES WALKER.

Veterinary Research Laboratories, Onderstepoort.

Some Observations in connection with the Immunization of Cattle against South African Redwater and Genuine Gallsickness (Anaplasmosis).

By JAMES WALKER, Veterinary Research Laboratories,
Onderstepoort.

DURING the year 1911 and succeeding years the writer immunized a number of imported susceptible cattle against South African redwater and genuine gallsickness (anaplasmosis), and an opportunity was afforded of closely following the reactions and noting the results of inoculation. On referring to the literature on anaplasmosis in cattle it will be noted that in the year 1893 Smith and Kilborne recorded the occurrence of "intra-corpuscular bodies," the so-called "coccus-like bodies, marginal points" in the blood of Texas cattle, and considered these to be a stage in the cycle of *P. bigeminum*, and associated their presence with a mild form of Texas fever. *Kolle and Turner*, in their report published in 1898, describe what are apparently similar bodies in bovines in South Africa; the former believed them to be young parasites.

Lignières published in 1900 the result of his investigations in connection with "La Tristza" or "Malaria bovine dans la Republique argentine," and apparently mistook the basophile granulations which appeared in the blood of animals with or recovering from this disease for the coccus-like bodies described by Smith and Kilborne, and concluded the latter represented an accident of preparation. *Knuth* in 1904 observed "Intra corpuscular" bodies in the blood of cattle in the "La Plata" States of South America, and held that they represented a stage in the cycle of *P. bigeminum*. *Dschunkowsky and Luhs*, in 1904, in an article entitled "Die Piroplasmosis der Rinder" recorded the occurrence of "marginal points" in Transeaucasian cattle and concluded they represented a form of *P. annulatum* (tropical piroplasmosis). In 1906 Theiler, as the result of some transmission experiments and observations, was inclined to think that the "coccus-like bodies" were connected with the development of *P. bigeminum*, but concluded the experiments were not sufficient to arrive at any definite conclusion.

In 1908 this investigator published the result of further experiments and concluded that the "chromatic bodies" or so-called "marginal points of cattle" represented a new genus of protozoon—the anaplasma—and were responsible for the genuine gallsickness of South African cattle; prior to then the generally accepted view was that it was a sequel of piroplasmosis. Since the pronouncement of Theiler, a lot of attention has been devoted to the subject, with the result that the literature includes quite a number of records of investigations and experiments carried out for the purpose of determining the nature of anaplasms, and their occurrence in bovines in various parts of the world has been recorded.

Bruce and his co-workers noted their occurrence in calves in Uganda, and considered they were cell inclusions or nuclear remnants. Lichtenheld in 1910 describes anaplasmosis as occurring in bovines in German East Africa.

Ollweg and Manteufel observed in the blood of cattle in German East Africa parasites, some of which had the character of *P. mutans*, others those of anaplasmas, and considered that the latter are stages in the cycle of piroplasmosis (*P. mutans*, *P. parvum*, and *P. bigeminum*).

Sieber studied the pathological changes which occurred in the blood of cattle that were put in experiment by Theiler, who isolated the anaplasma infection by means of ticks, and made morphological and biological investigations into the nature of *Anap. marginale*, and concluded that there is no doubt that the anaplasmas are of a parasitic nature, and compared them with formations which Prowazek placed in one group under the name of chlamydozoa. Lignières in his publication on "Bovine Anaplasmosis in the Argentine, 1914," concludes that anaplasmosis is endemic in cattle in certain regions in the northern Argentine, and is separable from *P. argentinum* and *P. bigeminum* with either, or both, of which it is generally associated in nature, and that animals immunized against *P. bigeminum* and *P. argentinum* are susceptible to anaplasmosis and vice versa. K. F. Meyer, in 1911, supported the conclusions of Smith and Kilborne, but further noted that the coccus-like bodies or marginal points also occur in fatal cases of so-called Texas fever, and in 1913 in an article entitled "Die Pernizioese Anaemie der Rinder" draws the following conclusions:—

- (1) That the parasitic nature or rather protozoal nature of *Anap. marginale* is by no means proved.
- (2) That the conception of Theiler that anaplasmosis is a distinct disease and separable from babesiosis is also not yet proved. This observer has noted that in many cases of Texas fever no temperature reaction to babesia occurs, and very often only after many hours of search of blood smears *P. bigeminum* is found, and cites an instance in which an Ayrshire cow which had never been in contact with ticks was infected with blood from a Texas fever animal, with the result that no reaction to *P. bigeminum* occurred; a reaction to anaplasmosis was however noted, and further, the blood of this Ayrshire cow produced in six calves an anaplasmosis infection. No mention is made of experiments having been made to ascertain whether the Texas animal was immune to redwater, and whether the Ayrshire cow and the six calves remained susceptible to Texas fever.

Springfeldt, in 1911, recorded the occurrence of anaplasmosis associated with *P. mutans* in the Cameroons.

Koidzumi, in 1912, as the result of the examination of a number of blood smears and of observations made in Formosa, is of opinion that *Babesia bigemina* at least takes the form of the marginal points in its life-cycle as believed by Smith and Kilborne. On the other hand, he believes that some of the "intra-corpuscular" bodies he noted may have been artefacts or abnormal inclusions in the corpuscule.

Carpano, in a preliminary note entitled "Anaplasmosis of the Bovidae in the Roman Campagna," drew attention to the occurrence of *Anaplasma marginale* in newly-imported cows and cows heavy in calf, but, later—1912—carried out some cultural experiments with *Babesia caballi* and *Nuttalia equi*, with the result that in cultures of the latter,

anaplasma-like bodies were observed, and the author concluded that the anaplasma ought to be considered as particular stages of diverse piroplasmosis, durable or latent forms, capable of (1) producing the same infection of piroplasmosis amongst susceptible animals; (2) reproducing anaplasma-like forms amongst animals possessing a certain degree of immunity; (3) conserving the virulency of the blood; (4) of determining a breakdown in immunity.

Veglia, at the Veterinary Research Laboratory, Onderstepoort, Pretoria, in 1914, with the object of separating *Anaplasma marginale* from similar intra-corporal bodies and other protozoa of a similar nature attempted the cultivation of *Anaplasma marginale* "in vitro," and from the results which he obtained concludes that—

1. Anaplasms grow and multiply in artificial media such as—

- (a) pure defibrinated blood, viz. blood from an animal infected with anaplasmosis;
- (b) salt solution (sodium chloride) as used by Nuttall for the cultivation of *Babesia canis*;
- (c) sodium citrate solution as used by Carpano for the cultivation of *Nuttallia equi*;
- (d) ordinary bouillon as used by Miyajima for the cultivation of *Babesia bovis*.

2. Basophile granulations and Jolly bodies, which are noted in anaemic blood, behave differently to anaplasms on cultivation, in as much as they did not increase in number and finally disappeared.

3. Normal blood of cattle and other animals not infected with anaplasms when cultivated in the way mentioned did not produce anaplasms.

4. In cultures made with pure anaplasmosis blood or anaplasma and babesia (mixed) blood, no form could be traced which would suggest a transmission of one to another, etc.

Lescazeaux et Picollo have noted the occurrence of anaplasmosis in the state of Saint Paul (Brazil), but consider that the sub-division into *Anaplasma marginale* and *centrale* is arbitrary. They found the two the more often mixed, and noted deaths from the centrale infection. The authors, however, seem to admit that a certain relation exists between the piroplasms and anaplasms, and draw the following conclusions:—

The distinction between the *Anap. marginale* and *centrale* should not be made. The writers think that the piroplasms are transferable into anaplasms.

Referring to the literature dealing with the occurrence of anaplasma-like bodies in other animals, it will be noted that Jowett in 1911 described anaplasma-like bodies in the blood of normal cats. K. F. Meyer confirms the observation of Jowett and refers to the number of similar marginal-like bodies which occur in the blood of cats dead of glanders or trypanosomiasis.

Balfour, in 1911, recorded an observation on the occurrence of anaplasma-like bodies in the blood of a donkey affected with biliary fever.

In 1913, *Dodd*, in an article entitled "Anaplasmosis or Jolly's Bodies," described bodies presenting morphological and staining characters identical with or similar to those possessed by anaplasms in the blood of wild animals, and wild animals in captivity, and suggests in view of the fact that the red corpuscles of the mouse deer are about one-third the size of those of the other species examined, and that in

these the bodies were correspondingly small, that this is further evidence tending to show that they are in reality remains of nuclei.

For Schilling Torgau the anaplasma-like bodies are degenerative and regenerative phenomena of the red cells, and occur in anaemia of animals other than bovines, e.g. guinea pigs, etc.

For Dias and Aragao (1) the anaplasmas are not protozoa, and they do not cause any disease. These conclusions were based on the result of injection of nitrobenzol or phenylhydrazine to cattle, cats, dogs, rabbits, rats, and guinea pigs which produced alterations in the blood, viz., *polychromasia*, *basophilia*, etc., and a decrease in the number of cells, as well as the appearance of coccus-like corpuscles situated on the margin or centre of the cell, and which cannot, according to the authors be distinguished from the anaplasmas of Theiler.

2. These investigators conclude that the anaplasma is a corpuscle of a haemic nature and a product of degeneration of the red blood cells.

3. The anaplasma is a globular degeneration in certain anaemias determined by a haemolytic substance.

4. The anaplasmosis described by Theiler is a clinical form of piroplasmosis. The anaplasmosis of mammalia occurs from diverse causes.

Joest and Jahnichen, under the heading "Einige Blutbefunde bei Osteomalacie der Pferde," draw attention to the occurrence of intra-corpuscular bodies in this disease.

1.—OBSERVATIONS IN CONNECTION WITH THE TRANSMISSION OF REDWATER.

During 1911 and the following years the writer inoculated a number of English imported susceptible cattle and a few South African cattle bred on a non-redwater farm with blood containing the double infection, viz., redwater and gallsickness. Some of the animals belonged to private owners and were imported for stud use, the remainder were immunized for the purpose of obtaining supplies of redwater-gallsickness vaccine. From the date of their landing, and on arrival at the station, measures were taken to prevent natural infection occurring. Prior to and after inoculation a daily blood smear was made and examined, and a temperature record kept. By these means it was possible to ascertain the result and control the redwater reaction if necessary by injection of trypan blue.

In some instances it was noted that the inoculation of redwater-gallsickness blood did not produce a redwater reaction. (No temperature reaction occurring and no *Piroplasma bigeminum* being seen in the blood smear, the animal remaining apparently healthy.) A reaction to anaplasmosis, however, invariably occurred.

In the report of the Government Veterinary Bacteriologist, Department of Agriculture, Transvaal, for the year 1908-09, page 8, reference is made to the inoculation of ten susceptible Sussex heifers by Stockman in London in November, 1908, with redwater blood (South African strain). Eight of these heifers likewise failed to react, and from the fact that they reacted to a subsequent inoculation it was concluded that the first inoculation conferred no immunity in the case of the above-mentioned eight animals.

Taking into consideration that the cattle under observation, belonging to private owners, had given no apparent reaction to redwater would, when handed over, run on redwater veld, it was decided

to ascertain whether they were actually immune before being returned, the method adopted being as follows:—

Blood was first collected from some of the apparent non-reactors and inoculated into a susceptible beast, and the former were then reinoculated with virulent blood.

The following are the details of the experiments:—

Experiment No. 1.

Bull A: Imported English Shorthorn (age 3 years), imported a few days previous to date of inoculation.

On the 9th August, 1911, inoculated subcutaneously with 10 c.c. redwater-gallsickness blood (*ex* Ox No. 9).

Result.

No temperature reaction to redwater. No *P. bigeminum* seen in the blood smears. On the 41st day after inoculation and on subsequent dates *Anaplasma marginale* noted. On the 71st day and on subsequent dates *P. mutans* noted.

Experiment No. 2.

Bull B: Friesland (age 2 years), imported a few days previous to date of inoculation. On the 9th August, 1911, inoculated subcutaneously with 10 c.c. redwater-gallsickness blood (*ex* Ox No. 9).

Result.

No temperature reaction to redwater. No *P. bigeminum* seen in the blood smears. On the 38th day after inoculation and on subsequent dates *Anaplasma marginale* noted. On the 52nd day and on subsequent dates *P. mutans* noted.

Experiment No. 3

Bull C: Imported English Shorthorn (age 3 years), imported a few days previous to date of inoculation. Inoculated on the 9th August, 1911, with 10 c.c. redwater-gallsickness blood (*ex* Ox No. 9).

Result.

No apparent temperature reaction to redwater. No *P. bigeminum* noted in blood smears. On the 38th day after inoculation and on subsequent dates *Anaplasma marginale* noted. Animal died on the 45th day from anaplasmosis.

Experiment No. 4.

Heifer D: Imported English Shorthorn (age 3 years), imported a few days previous to date of inoculation. Inoculated on the 9th August, 1911, with 10 c.c. redwater-gallsickness blood (*ex* Ox No. 9).

Result.

No temperature reaction to redwater. On the 38th day and on subsequent dates *Anaplasma marginale* observed. On the 72nd day and on subsequent dates *P. mutans* seen.

Experiment No. 5.

Bull X: Shorthorn, Colonial bred (age 1 year), bred on a non-redwater farm, inoculated on the 16th January, 1913, with 10 c.c. redwater-gallsickness blood (*ex* Hereford heifer No. 148).

Result.

No temperature reaction to redwater. No *P. bigeminum* noted in the blood smears. On the 20th day after inoculation and on subsequent dates *Anaplasma marginale* observed. On the 43rd day and on subsequent dates *P. mutans* seen.

Subsequent to the above experiments some imported Hereford heifers non-immune to South African redwater and gallsickness arrived at the Grahamstown Laboratory for the purpose of replacing the Africander oxen which had been in use there for some considerable time for supplying redwater and gallsickness blood, and whose blood contained the double infection, viz., redwater and gallsickness (*Anap. marginale*).

The immunization of these heifers was proceeded with as follows:—One of the heifers was inoculated with blood from one of the above-mentioned Africander oxen and bled when reacting to redwater, and the blood inoculated to another of the clean heifers, the object being to establish the strain of redwater existing in the ox and exclude the *Anaplasma marginale* infection, *Anaplasmosis* (variety *centrale*) to be substituted for *Anaplasma marginale* by inoculation of the heifers with blood containing a pure infection of the former.

A further opportunity was thus afforded of noting the results of inoculation of redwater-gallsickness blood to imported susceptible cattle. In the following instances no reaction to redwater was observed.

Experiment No. 6.

Hereford No. 281: Imported English Hereford (age 2 years), imported about the 12th July, 1912, and kept tick free since arrival. On the 9th September, 1912, inoculated with 10 c.c. redwater-gallsickness blood (*ex* Ox No. 40).

Result.

From the 19th to the 25th day temperature irregular, maximum 103; irregular temperature attributed to oestrus, a hæmorrhagic discharge being noticed from the vulva on the 21st day. No *P. bigeminum* noted in blood smears. On the 44th day after inoculation and on subsequent dates *Anap. marginale* noted. *P. mutans* noted from the 61st day.

Experiment No. 7.

Hereford heifer No. 283: Imported English Hereford (age 2 years), imported about the 12th July, 1912, and kept tick free since arrival. On the 5th December, 1912, inoculated with 10 c.c. blood (*ex* Hereford heifer No. 282). Heifer No. 282 was reacting to redwater, and *P. bigeminum* were fairly frequent in the blood smears when the blood was collected on the 5th December, 1912 (blood was collected from 282 and inoculated to 283 before the reaction to anaplasmosis occurred in the former).

Result.

No apparent temperature reaction to redwater. No reaction to anaplasmosis. No reaction to *P. mutans*.

Résumé.—In six instances (see experiments Nos. 1, 2, 3, 4, 5, and 6) in which susceptible cattle were inoculated with redwater-gallsickness blood no reaction to redwater resulted, and no *P.*

bigeminum were seen in the blood smears. In one instance (see experiment No. 7) an animal which was inoculated with blood collected at the time of the redwater reaction, and prior to the anaplasmosis reaction and in which *P. bigeminum* were fairly frequent in the peripheral circulation, failed to react to redwater and did not react to anaplasmosis and *P. mutans*.

The following experiments were carried out to note whether the blood of animals which had not shown a temperature reaction, and in which *P. bigeminum* did not appear in the blood smears when inoculated with redwater-gallsickness blood, would transmit redwater to susceptible cattle:—

Experiment No. 8, Non-immune Calf No. 154, Colonial Bred.

Inoculated on the 19th September, 1911, with 10 c.c. blood of Bull B. (Bull B was reacting to anaplasmosis when the blood was collected.)

Result.

On the 10th day after inoculation and on subsequent dates *Anaplasma marginale* seen in blood smears. On the 53rd day and on subsequent dates *P. mutans* recorded. No temperature reaction to redwater resulted and no *P. bigeminum* were seen in the blood smears.

Experiment No. 9, Non-immune Calf No. 153, Colonial Bred.

Inoculated on the 19th September, 1911, with 10 c.c. blood of Bull C. (Bull C was reacting to anaplasmosis when the blood was collected.)

Result.

On the 10th day after inoculation and on subsequent dates *Anaplasma marginale* seen in the blood smears. No temperature reaction to redwater resulted, and no *P. bigeminum* were observed in the blood smears. Animal died on the 54th day after inoculation.

Résumé.—In two instances (see experiments Nos. 8 and 9) the inoculation of susceptible cattle with blood of an animal which showed no reaction to redwater, but which reacted to anaplasmosis, when inoculated with redwater-gallsickness blood did not produce a redwater reaction, but anaplasmosis resulted.

Since the blood of animals which showed no reaction to redwater when inoculated with redwater-gallsickness blood did not produce a redwater reaction, when inoculated to susceptible cattle (experiments Nos. 8 and 9), the former were tested on their immunity to redwater, and for this purpose they were reinoculated with blood of a different immune animal to that previously used.

Experiment No. 10.

Bull A (see previous experiment No. 1): Reinoculated on the 31st October, 1911, with 10 c.c. redwater-gallsickness blood (*ex* Hereford heifer No. 148).

Result.

On the 12th day after temperature reaction to redwater commenced temperature remained elevated for some days, maximum 105.4. *P. bigeminum* noted in blood smears during reaction.

Experiment No. 11.

Bull B (see previous experiment No. 2): Reinoculated on the 31st September, 1911, with 10 c.c. redwater-gallsickness blood (*ex* Hereford heifer No. 148).

Result.

On the 18th day after inoculation temperature reaction to redwater commenced, maximum 107, temperature normal on the 21st day.

A clean calf not being available to ascertain whether the blood of heifer D would transmit redwater, and as it was desirous to return the animal to the owner as soon as possible, heifer D was reinoculated with blood of a different immune animal to that previously used.

Experiment No. 12.

Heifer D (see previous experiment No. 4): Reinoculated on the 4th November, 1911, with 5 c.c. redwater-gallsickness blood (*ex* Hereford heifer No. 148).

Result.

On the 10th day after inoculation temperature reaction to redwater commenced. *P. bigeminum* seen in blood smears during the reaction.

Résumé.—In two instances (see experiments Nos. 10 and 11) cattle which had failed to react to redwater after inoculation with redwater-gallsickness blood (see experiments Nos. 1 and 2), and whose blood did not transmit redwater to a susceptible animal (see experiments Nos. 8 and 9), when reinoculated with blood from a different immune animal to that previously used gave a typical redwater reaction, and *P. bigeminum* appeared in the blood smears.

In one instance (see experiment No. 12) an animal which had failed to react to redwater after inoculation with redwater-gallsickness blood (see experiment No. 4) when reinoculated with blood from a different immune animal gave a typical redwater reaction and *P. bigeminum* appeared in the blood smears.

No clean calves being available to ascertain whether the blood of X and 281 and 283 would transmit redwater, it was decided to reinoculate these animals for the purpose of testing them on their immunity.

The following are the results:—

Experiment No. 13.

Bull X (see previous experiment No. 5): Reinoculated on the 24th February, 1913, with 10 c.c. virulent blood (*ex* Hereford heifer No. 282).

Result.

On the 12th day *P. bigeminum* seen in the smears, maximum temperature 105 (temperature curve irregular for some days previous to and after inoculation, due to the *Anaplasmosis* and *P. mutans* infections from the previous inoculation, parasites of which were observed in the smears).

Experiment No. 14.

Hereford heifer No. 281 (see previous experiment No. 6): Reinoculated on the 22nd November, 1912, with 10 c.c. redwater *P. mutans* blood (*ex* cattle 1918, Pretoria).

Result.

No *P. bigeminum* seen in blood smears. Temperature curve irregular for some days previous to and after inoculation due to anaplasmosis and *P. mutans* reactions from the previous inoculation, parasites of which were observed in the smears.

Experiment No. 15.

Hereford heifer No. 283 (see previous experiment No. 7): Re-inoculated on the 13th February, 1913, with 10 c.c. redwater blood and *P. mutans* (ex cattle 1918, Pretoria).

Result.

From the 12th to 15th day after inoculation temperature elevated, maximum 106; smears were carefully examined, but no *P. bigeminum* seen.

Résumé.—An animal which had been previously inoculated with blood of an immune redwater-gallsickness animal (see experiment No. 5), and had not shown a reaction to redwater, when reinoculated with blood from another immune animal gave a temperature reaction, and *P. bigeminum* appeared in the blood smears (see experiment No. 13).

An animal which had been previously inoculated with redwater-gallsickness blood, and which did not apparently react to redwater (see experiment No. 6), when reinoculated with blood of another animal immune, did not give a temperature reaction to redwater and no *P. bigeminum* were observed in the blood smears (see experiment No. 14).

An animal which had been previously inoculated with blood collected from a beast which was reacting to redwater, and in which redwater parasites were frequent, no apparent reaction to *P. bigeminum*; *P. bigeminum* anaplasmosis or *P. mutans* resulting (see experiment No. 7). When reinoculated with blood of another immune animal containing *P. bigeminum* and *P. mutans* infections gave a temperature reaction to redwater, but no redwater parasites were seen in the smears before, during, and after the reaction. (See experiment No. 16.) *P. mutans* appeared from the 54th day.

Since a susceptible animal which was inoculated with redwater-gallsickness blood showed no reaction to redwater, and when reinoculated with blood of another animal did not react (see experiments Nos. 6 and 14), it was decided to continue reinoculating the animal and to carry out some transmission experiments in order to ascertain whether the blood of an animal which showed no reaction would transmit redwater to susceptible cattle.

Experiment No. 16, Immunity Test.

Hereford heifer No. 281 (see previous experiments Nos. 6 and 14), reinoculated on the 13th February, 1913, with 10 c.c. blood (ex heifer 1918, Pretoria).

Result.

Anaplasma marginale and *P. mutans* were occurring in the smears before and after inoculation; irregular temperatures noted before and after inoculation probably due to these infections. No *P. bigeminum* seen in the smears.

Experiment No. 17.

To note whether the blood of Hereford heifer 281 would transmit redwater to a susceptible animal. Non-immune calf 305 inoculated on the 6th March, 1913, with 10 c.c. blood of Hereford heifer 281. (*P. mutans* was noted in the blood smears which were made when the blood was collected for inoculation.)

Result.

The temperature was elevated on the seventh day, maximum 105. No reaction to redwater and no *P. bigeminum* seen in the blood smears. On the 26th day and on subsequent dates, *P. mutans* noted. On the 29th day and on subsequent days, *Anaplasma marginale* noted.

Experiment No. 18.

To note the effects of reinoculation with blood of Hereford heifer 281. On the 7th April, 1913, calf 305 reinoculated with blood of Hereford heifer 281.

Result.

From the 4th to the 15th day temperature elevated, maximum 105. *Anaplasma marginale* were seen in the smears of calf 305 on the day the animal was inoculated; elevation of temperature was probably due to this infection. No *P. bigeminum* seen in the smears.

Experiment No. 19.

To note whether reinoculation would cause a reaction to redwater. Calf 305 reinoculated on the 13th May, 1913, with 10 c.c. blood of Hereford heifer 284 (same strain as that used for the two previous inoculations).

Result.

No reaction to redwater; no *P. bigeminum* seen in the smears.

Experiment No. 20.

To note whether blood of another immune animal would produce a reaction to redwater. On the 5th August, 1913, calf 305 reinoculated with 10 c.c. blood (*ex* Hereford heifer 286).

Result.

On the 6th and 7th days marked temperature reaction to redwater. Maximum temperature 105.8, and *P. bigeminum* noted in smears during the reaction.

Experiment No. 21.

To ascertain whether blood of another immune animal would cause cattle 281 to react to redwater. Cattle 281 reinoculated on the 11th July, 1913, with 10 c.c. blood (*ex* Hereford heifer 284).

Result.

From the 11th to the 15th days temperature reaction, maximum 105.8. Absent from headquarters when the temperature was elevated, and no smears were collected, but smears collected on the 20th to 24th day showed polychromatic cells and blood changes probable sequel of redwater.

Experiment No. 22.

To note effects of reinoculation with blood of another immune animal. Reinoculated on the 8th September, 1913, with 10 c.c. blood (ex Hereford heifer 286).

Result.

On the 8th to 10th days, temperature elevated, maximum 106.6, and *P. bigeminum* frequent during the reaction.

Résumé.

A susceptible animal which gave no reaction to redwater when inoculated with redwater-gallsickness blood (see experiment No. 6) showed an irregular temperature when inoculated with blood of another immune animal (see experiment No. 14) which was attributed to the gallsickness and *P. mutans* infections which resulted from the previous inoculation, but no characteristic redwater reaction was observed or *P. bigeminum* seen in the blood smears. Reinoculation with blood from the same animal as used in the previous experiment gave likewise a negative result (see experiment No. 16). The animal when again inoculated with blood from another (see experiment No. 21) immune animal, but of the same strain as that used for the original inoculation, showed a characteristic temperature reaction to redwater. No smears were collected during the period the temperature was elevated owing to absence from headquarters, but the presence of polychromatic cells on the 20th to the 24th days pointed to the reaction being due to redwater. When the animal was again inoculated with blood of a different strain to those used for the previous inoculations, a characteristic redwater reaction occurred, and *P. bigeminum* were seen in the smears (see experiment No. 22). Blood collected from the animal before it was inoculated with the blood which produced the reaction and injected into a susceptible calf caused a temperature reaction, but no *P. bigeminum* were seen in the smears (see experiment No. 17). The susceptible calf was reinoculated twice with blood of same strain, and again no reaction was noted (see experiments Nos. 18 and 19), but when reinoculated with blood of another immune animal a characteristic rise of temperature resulted and *P. bigeminum* were observed in the smears (see experiment No. 20).

Experiments to note whether an animal (Hereford heifer 283) which did not react to redwater when injected with blood showing *P. bigeminum* fairly frequent and which apparently reacted to a subsequent inoculation (see experiments Nos. 7 and 15) would again react if reinoculated.

Experiment No. 23.

Hereford heifer 283 (see previous experiments Nos. 7 and 15), reinoculated on the 31st May, 1913, with 10 c.c. blood of cattle 2655, Pretoria.

Result.

No temperature reaction to *P. bigeminum* and no *P. bigeminum* seen in the smears.

Experiment No. 24.

To ascertain whether the blood of another immune animal would transmit redwater. Reinoculation on the 11th July, 1913, with 10 c.c. blood of Hereford heifer No. 284 (redwater-gallsickness blood).

Result.

No temperature reaction to redwater; no *P. bigeminum* seen in the smears. On the 34th to the 37th days after temperature elevated, *Anaplasma marginale* very frequent in the smears. Temperature reaction attributed to this infection.

Résumé.

Reinoculation of an animal (which had failed to react to redwater when inoculated with blood collected at the time of the redwater reaction) with redwater and *P. mutans* blood produced a temperature reaction to redwater as well as a *P. mutans* infection, but no reaction to anaplasmosis; reinoculation with blood containing redwater and *P. mutans* infection again resulted in no anaplasmosis reaction. When the animal was inoculated with redwater-gallsickness blood a reaction to anaplasmosis resulted.

On referring to the foregoing experiments, it will be noted that (1) susceptible cattle which did not react to redwater when inoculated with redwater-gallsickness blood invariably reacted to anaplasmosis (experiments Nos. 1, 2, 3, 4, 5, and 6).

(2) Their blood produced in susceptible cattle anaplasmosis but not redwater (see experiments Nos. 8, 9, and 17).

(3) Reinoculation of animals which failed to react to redwater after inoculation of redwater-gallsickness blood resulted in a reaction to redwater (see experiments Nos. 10, 11, 12, 13, and 21).

(4) Blood collected from an animal which was reacting to redwater as the result of inoculation with redwater-gallsickness blood and, consequently, before the anaplasmosis reaction commenced, did not produce in a susceptible animal (one experiment) redwater, and no reaction to anaplasmosis occurred (see experiment No. 7).

(5) The latter when reinoculated with redwater blood reacted to redwater but not to anaplasmosis (experiment No. 15).

(6) Reinoculation with redwater blood again failed to produce a reaction to anaplasmosis (experiment No. 23).

(7) Reinoculation with redwater-gallsickness blood produced a reaction to anaplasmosis (experiment No. 24).

(8) An animal which has reacted to *P. bigeminum* may again react when inoculated with blood of another immune animal (different strain); compare experiments 21 and 22

Table 1 shows results of first and subsequent inoculations and immunity tests.

After returning to headquarters, Pretoria, the writer immunized a further lot of imported cattle against redwater and gallsickness which were to be utilized for the supply of redwater-gallsickness as well as some Colonial-born calves, and, in order to reduce the risk of tick-infection to a minimum, the former were railed in clean trucks immediately after debarkation to the laboratory siding, and, on arrival, were put in tick-free stables. The Colonial-born calves were brought to the laboratory shortly after birth and likewise kept in tick-free stables.

From the date of arrival on the station both lots of animals were put under observation, a temperature record kept, and blood smears collected and examined daily from the date of inoculation.

During the period 29th October, 1913, to 8th March, 1915, twenty imported cattle and one susceptible calf born of an imported Hereford heifer on the station, as well as fourteen Colonial-born calves, were

put in experiment, the latter, as stated above, serving as controls. The details of each experiment are given in the tables marked 2, 3, and 4.

Of the twenty imported cattle which were inoculated with redwater-gallsickness blood, nineteen gave a temperature reaction to redwater, and *P. bigeminum* were noted in the smears during or shortly after the temperature reaction (each of these nineteen animals reacted as well to anaplasmosis).

(One of the imported cattle, No. 3128, showed no temperature reaction to redwater, and no *P. bigeminum* were seen. A reaction to *Anaplasma marginale* commenced on the 18th day after inoculation. (Death occurred on the 25th day from anaplasmosis.) Calf 2953 (born of imported Hereford heifer) gave a temperature reaction to redwater (but no *P. bigeminum* were observed in the smears) and reacted to anaplasmosis.

Of the fourteen control calves which were inoculated with redwater-gallsickness blood—

Eight reacted to redwater, and redwater parasites were noted in the smears during or shortly after the temperature reaction; each of the eight reacted to anaplasmosis.

Four, viz., 3258, 3260, 3272, and 3305 gave a temperature reaction to redwater, but no *P. bigeminum* were observed in the smears during or shortly after the temperature reaction; in two of these, viz., 3258 and 3305, *P. bigeminum* were noted in the smears some days after the reaction. All four reacted to anaplasmosis.

Of the remaining two, heifer 3279 showed no temperature reaction to redwater, and no *P. bigeminum* were seen in the smears. *Anaplasma marginale* was recorded in the smears from the 19th day after inoculation.

Calf 3344 gave a slight temperature reaction to redwater. Anaplasmosis was noted on the 16th day and *P. bigeminum* on the 24th day after inoculation.

As the result of the above experiments and observations, the following conclusions were arrived at.

Redwater Transmission.

1. The inoculation of susceptible cattle with redwater-gallsickness blood did not always result in a reaction to redwater. (No rise of temperature occurred and no *P. bigeminum* were noted in the smears. Animal remained apparently healthy.)

2. The blood of an animal which gave no reaction to redwater when inoculated with redwater-gallsickness blood does not transmit redwater to susceptible cattle.

3. Susceptible animals which failed to react to redwater after inoculation are susceptible to redwater.

4. The inoculation of a susceptible animal with blood which was collected from a beast when reacting to redwater, and in which *P. bigeminum* parasites were frequent does not always produce a redwater reaction (one experiment), and the animal remains susceptible to redwater.

5. An animal which has reacted to a strain of redwater may again react when inoculated with blood of a different strain.

6. The redwater reaction may manifest itself in a rise of temperature without the appearance of *P. bigeminum* in the smears during or shortly after the temperature reaction.

2.—OBSERVATIONS IN CONNECTION WITH THE TRANSMISSION OF ANAPLASMOSIS.

(a) The invariability of transmission of anaplasmosis by means of inoculation of redwater-gallsickness blood.

(b) The mutability of anaplasmosis (variety centrale).

(a) *The Invariability of Transmission of Anaplasmosis by means of Inoculation of Redwater-Gallsickness Blood.*

On referring to the foregoing experiments it will be noted (1) that the inoculation of susceptible cattle with redwater-gallsickness blood did not always produce a reaction to redwater; a reaction to anaplasmosis however invariably occurred.

(2) The blood of animals which failed to react to redwater after inoculation with redwater-gallsickness blood produced in susceptible cattle anaplasmosis but not redwater.

(3) Blood collected from an animal which had been inoculated with redwater-gallsickness blood during the redwater reaction and thus before the anaplasma infection appeared did not produce anaplasmosis in a susceptible animal.

(4) The shortest period in which *Anaplasma marginale* appeared after inoculation was 20 days, longest period 44 days, average period 31 days. For *A. centrale* the shortest period was 16 days, longest 47, average 32 days.

(b) *The Mutability of Anaplasma Centrale.*

As the result of further observation experiments (First Report of the Director of Veterinary Research, 1911, page 45), Theiler classified the anaplasmata into two varieties, viz., the *Anap. marginale* and the *Anap. marginale* (variety centrale), and introduced a method of immunization against the former based on the fact that the blood of an animal which has reacted to *Anaplasma centrale* when inoculated to a susceptible animal transmits the centrale infection and protects against *Anaplasma marginale* (proper), the latter being more virulent, and, in the majority of cases, responsible for deaths occurring from genuine gallsickness contracted naturally. In order to obtain supplies of gallsickness vaccine, imported cattle are inoculated with the centrale infection, and since it is the rule to find that both gallsickness and redwater co-exist on the same farm, they are also immunized against the latter, and their blood containing the double infection supplied for immunization purposes.

During the course of the last year or two a number of imported susceptible cattle were immunized for this purpose, and it was observed that the inoculation of blood of an animal immune to anaplasma (variety centrale) did not always result in a pure centrale infection being transmitted but produced in some cases a pure marginale infection. Taking into consideration that blood containing the centrale infection produces only a mild anaplasmosis reaction whilst that containing *Anaplasma marginale* proper causes a high percentage of mortality, it is of importance to note whether a mutation from centrale to marginale occurs in passage through animals intended for use for the supply of vaccine. The genealogical chart shows the mutations which occurred.

Résumé.

The observations extended over a period of five years and a few days, namely, from 28th February, 1910, to the 8th March, 1915;

during this time forty-three cattle were inoculated, and in thirty-five of these the experiment was controlled by the writer.

In the first series of inoculations no mutation occurred during the passage through eight successive generations, viz. from the 28th February, 1910, to 30th December, 1913.

An animal inoculated approximately eight months later than the latter date with blood of the seventh generation and two animals inoculated with blood of the eighth generation reacted to *Anaplasma marginale*.

In the second series of inoculations, whereas no mutation occurred in some cases in passage through successive generations, viz. from the 28th February, 1910, to the 8th March, 1915, two cattle inoculated with blood of an eighth generation animal reacted to *Anaplasma marginale*.

In the third series of inoculations, whereas centrale blood of the sixth generation produced a centrale infection, blood obtained from the same animal, approximately 5 and 9 months later, produced a marginale infection and the inoculation of the blood of two imported animals which reacted to *Anap. centrale* resulted in a marginale infection.

Conclusion.

A mutation from *Anap. centrale* to *Anap. marginale* may occur in passage through a susceptible animal.

The determination of the type is probably according to the Mendelian law, the marginale being the dominant—the centrale the recessive type.

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TABLE SHOWING RESULTS OF FIRST AND SUBSEQUENT INOCULATIONS AND IMMUNITY TESTS.

Table No. 1.

1ST INOCULATION.				IMMUNITY TEST.			2ND INOCULATION.			3RD INOCULATION.			IMMUNITY TEST.			IMMUNITY TEST.			4TH INOCULATION.			5TH INOCULATION.		
Animal.	1st Inoculation.	Date.	Result.	Immunity test to note whether blood infective.	Date.	Result.	2nd Inoculation.	Date.	Result.	3rd Inoculation.	Date.	Result.	Immunity Test.	Date.	Result.	Immunity Test.	Date.	Result.	4th Inoculation.	Date.	Result.	5th Inoculation.	Date.	Result.
Bull A. (Imported.)	10 c.c. blood. Subcutaneously ex Ox 9. (Redwater-gallsickness blood)	9/8/11	Negative to <i>P. bigemum</i> . Reacted to <i>Anap. marginale</i> and <i>P. mutans</i>	None made. No clean cattle available	—	—	10 c.c. blood ex A. 148. (Redwater-gallsickness blood)	30/10/11	Reaction to <i>P. bigm.</i> and animal returned to owner	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Bull B. (Imported.)	10 c.c. blood. Subcutaneously ex Ox 9. (Redwater-gallsickness blood)	9/8/11	Negative to <i>P. bigemum</i> . Reacted to <i>Anap. marginale</i> and <i>P. mutans</i>	Calf 154 inoculated subcutaneously with 10 c.c. blood of Bull B. (Anaplasmosis blood)	19/9/11	Negative to <i>P. bigm.</i> On the 10th day after inoculation, reaction commenced to <i>Anap. marginale</i> . On the 55th day and on subsequent dates <i>P. mutans</i> recorded	10 c.c. blood ex heifer 148. (Redwater-gall sickness blood)	31/10/11	Reaction to <i>P. bigm.</i> and animal returned to owner	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Bull C. (Imported.)	10 c.c. blood. Subcutaneously ex Ox 9. Redwater-gallsickness blood	9/8/11	Negative to <i>P. bigemum</i> . On the 38th day after inoculation reaction to <i>Anap. margle</i> commenced. Animal died on the 45th day from anaplasmosis	Calf 153 inoculated subcutaneously with blood of Bull C. (Anaplasmosis blood)	19/9/11	Negative to <i>P. bigemum</i> . On the 10th day after inoculation reaction to <i>Anap. marginale</i> commenced	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Heifer D. (Imported.)	10 c.c. blood. Subcutaneously ex Ox 9. (Redwater-gallsickness blood)	9/8/11	Apparently negative to <i>P. bigm.</i> On the 28th day after inoculation reaction to <i>Anap. margle</i> <i>P. mutans</i> first noted on 72nd day	None made. No clean cattle available	—	—	10 c.c. blood subcutaneously ex H. 148. (Redwater-gallsickness blood)	4/11/11	Reaction to <i>P. bigm.</i> and animal returned to owner	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Bull X. (Colonial bred.)	10 c.c. blood ex H. 148. (Redwater-gallsickness blood)	16/1/13	Negative to <i>P. bigm.</i> Reacted to <i>Anap. marginale</i> and <i>P. mutans</i>	None made. No clean cattle available	—	—	10 c.c. blood subcutaneously ex H. 282. (Redwater-gallsickness blood)	24/2/13	Reacted to <i>P. bigm.</i> and returned to owner	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
H. 281. (Imported.)	10 c.c. blood, ex Ox 40. (Redwater-gallsickness blood)	9/9/12	Negative to <i>P. bigm.</i> Reacted to <i>Anap. marginale</i> and <i>P. mutans</i> . <i>Anap. marginale</i> noted from the 4th day and <i>P. mutans</i> from the 61st day after inoculation	None made. No clean calves available	—	—	10 c.c. blood ex 1918. (Redwater and <i>P. mutans</i> blood)	22/11/12	Negative to <i>P. bigm.</i>	10 c.c. blood ex cattle 1918 (Pretoria). (Redwater - <i>P. mutans</i> blood)	13/2/13	No redwater reaction	Calf 305 inoculated subcutaneously with 10 c.c. blood ex cattle 281. (Redwater-gallsickness blood)	6/3/13	Reacted to <i>Anap marginale</i> and <i>P. mutans</i> . (Negative to redwater.)	Calf 305 inoculated with 10 c.c. blood ex cattle 286. (Redwater-gallsickness blood)	5/8/13	Reacted to redwater	10 c.c. blood ex cattle 284. (Redwater-gallsickness blood)	11/7/13	Reacted to redwater	10 c.c. blood ex 286. (Redwater-gallsickness blood)	8/9/13	Reacted to redwater.
													Calf 305 reinoculated with 10 c.c. blood ex cattle 281. (Redwater - gallsickness blood)	7/4/13	No reaction to <i>P. bigm.</i>									
													Calf 305 reinoculated with 10 c.c. blood of Hereford H. 281. Redwater-gallsickness blood)	13/5/13	Negative to redwater									
H. 283. (Imported.)	10 c.c. blood, ex cattle 282. <i>P. bigm.</i> frequent in smears when blood collected for the inoculation. (Redwater blood)	5/12/12	Apply negative to <i>P. bigm.</i>	None made. No clean cattle available	—	—	10 c.c. blood ex 1918. (Redwater and <i>P. mutans</i> blood)	13/2/13	Temperature reaction to <i>P. bigm.</i> No <i>P. bigm.</i> seen in smears. Reaction to <i>P. mutans</i>	10 c.c. blood ex 2653. (Redwater - <i>P. mutans</i> blood)	31/5/13	No redwater reaction	—	—	—	—	—	—	Ex cattle 284. (Redwater and gallsickness blood)	11/7/13	No reaction to Redwater. Reacted to anaplasmosis.	—	—	

Table No. 2.

Expt. No.	No. of Animal in Expt.	Breed.	Date Inoculated.	Inoculated subcutaneously with.	Result.	Remarks.
1	2656	Hersford heifer (imported)	25/8/12	10 c.c. blood <i>ex</i> 1917. Redwater and gallsickness blood	Reacted to redwater. From the 34th day reaction to <i>Anap. centrale</i>	Well marked temperature reaction to redwater from the 7th-11th day. No <i>P. bigeminum</i> seen. Ansoy-tosis from the 5/9/12-12/9/12.
2	2953	Hersford heifer calf (born of 2656)	29/10/13	10 c.c. blood <i>ex</i> 2656. Redwater and gallsickness blood	From the 30th day reaction to <i>Anap. centrale</i>	Temperature reaction to redwater. No <i>P. bigeminum</i> seen. Blood of this animal transmitted redwater and gallsickness to cattle No. 3127.
3	3127	Aberdeen Angus (imported)	30/12/13	10 c.c. blood <i>ex</i> 2953. Redwater and gallsickness blood	Reacted to <i>P. bigeminum</i> . From the 23rd day reaction to <i>Anap. centrale</i>	Temperature reaction from the 15th-17th day after inoculation. <i>P. bigeminum</i> noted in smears during reaction.
4	3305	Colonial bred calf (Control)	12/5/14	10 c.c. blood <i>ex</i> 3127. Redwater and gallsickness blood	Reacted to <i>P. bigeminum</i> . From the 25th day reaction to <i>Anap. marginale</i>	Temperature reaction to redwater. No <i>P. bigeminum</i> seen during the reaction. <i>P. bigeminum</i> noted on the 33rd day after inoculation.
5	3257	Colonial bred calf (Control)	9/5/14	10 c.c. blood <i>ex</i> 3127. Redwater and gallsickness blood	Reacted to <i>P. bigeminum</i> . On the 36th day <i>Anap. marginale</i> noted	Temperature reaction to redwater. <i>P. bigeminum</i> noted during the reaction.
6	3417	Colonial bred calf (Control)	21/7/14	10 c.c. blood <i>ex</i> 2953. Redwater and gallsickness blood	Reacted to <i>P. bigeminum</i> . On the 27th day <i>Anap. marginale</i> noted	Temperature reaction to redwater. <i>P. bigeminum</i> noted on 9th day.

Table No. 3.

No. of Expt.	No. of Animal in Expt.	Breed.	Date Inoculated.	Inoculated subcutaneously with.	Result.	Remarks.
1	2655	Imported Hereford heifer	26/8/12	10 c.c. blood <i>ex</i> 1913. gallsickness blood	Reacted to redwater. From the 31st day <i>Anaplasma centrale</i> noted	Temperature reaction to redwater marked and <i>P. bigenitum</i> noted on the 12th and 13th days after inoculation.
2	3088	Colonial bred calf (Control)	29/10/13	10 c.c. blood <i>ex</i> 2655. Redwater and gallsickness blood	Reacted to <i>P. bigenitum</i> . From the 47th day <i>Anaplasma centrale</i> noted	Well marked temperature reaction to red- water. <i>P. bigenitum</i> noted on the 19th and 20th days after inoculation.
3	3420	Colonial bred calf (Control)	21/7/14	10 c.c. blood <i>ex</i> 3088. Redwater and gallsickness blood	Reacted to redwater. From the 39th to 66th day <i>Anap. centrale</i> noted	Temperature reaction to redwater. <i>P.</i> <i>bigenitum</i> noted on the 17th day after inoculation.
4	3432	Imported Aberdeen Angus	4/9/14	10 c.c. blood <i>ex</i> 3420. Redwater and gallsickness blood	Reacted to redwater. From the 25th day <i>Anap. centrale</i> and <i>marginalis</i> noted	Temperature reaction to redwater. <i>P.</i> <i>bigenitum</i> in smears of 10th, 12th, and 13th days.
5	3436	Imported Aberdeen Angus	11/9/14	10 c.c. blood <i>ex</i> 3088. Redwater and gallsickness blood	Reacted to redwater. From the 34th day <i>Anap. centrale</i> noted	Temperature reaction to redwater. <i>P.</i> <i>bigenitum</i> noted on the 18/9/14.
6	3438	Colonial bred calf (Control)	11/9/14	10 c.c. blood <i>ex</i> 3088. Redwater and gallsickness blood	Reacted to redwater. From the 38th day <i>Anap. centrale</i> noted	Temperature reaction to redwater. <i>P.</i> <i>bigenitum</i> noted on the 8th day.
7	3544	Colonial bred calf (Control)	30/9/14	10 c.c. blood <i>ex</i> 3432. Redwater and gallsickness blood	From the 16th day <i>Anap. centrale</i> noted	No temperature reaction to redwater.
8	3466	Shorthorn.....	26/10/14	10 c.c. blood <i>ex</i> 3432. Redwater and gallsickness blood	Reacted to redwater. From the 21st day <i>Anap. marginalis</i> noted	Temperature reaction to redwater. <i>P.</i> <i>bigenitum</i> noted on the 7th day
9	3437	Aberdeen Angus...	26/10/14	10 c.c. blood <i>ex</i> 3432. Redwater and gallsickness blood	Reacted to redwater. From the 22nd day <i>Anap. marginalis</i> noted	Temperature reaction to redwater. <i>P.</i> <i>bigenitum</i> noted on the 2/11/14.

Table No. 3 (continued).

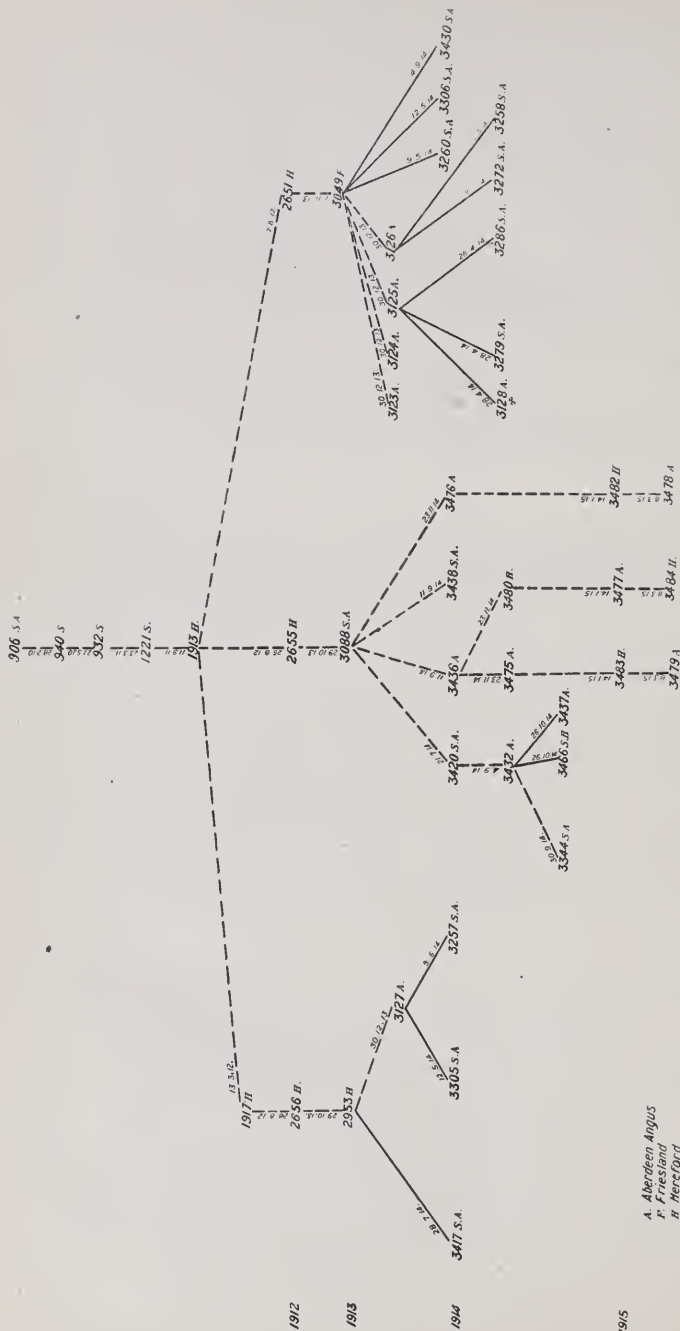
No. of Expt.	No. of Animal in Expt.	Breed.	Date Inoculated.	Inoculated subcutaneously with.	Result.	Remarks.
10	3476	Aberdeen Angus...	23/11/14	10 c.c. blood <i>ex</i> 3088. Redwater and gallsickness blood	Reacted to redwater. From the 24th day <i>centrale</i> noted	Temperature reaction to redwater. <i>P. bigenimum</i> noted on the 1/12/14.
11	3475	Aberdeen Angus...	23/11/14	10 c.c. blood <i>ex</i> 3436. Redwater and gallsickness blood	Reacted to redwater. From the 24th day reaction to <i>Anap. centrale</i>	Temperature reaction to redwater. <i>P. bigenimum</i> noted on the 1/12/14.
12	3480	Hereford heifer...	23/11/14	10 c.c. blood <i>ex</i> 3436. Redwater and gallsickness blood	Reacted to redwater. From the 24th day reaction to <i>Anap. centrale</i>	Temperature reaction to redwater. <i>P. bigenimum</i> in smears of the 30/11/14.
13	3482	Hereford heifer.....	14/1/15	10 c.c. blood <i>ex</i> 3476. Redwater and gallsickness blood	Reacted to redwater. From the 35th day <i>Anap. centrale</i> noted	Temperature reaction to redwater. <i>P. bigenimum</i> noted from the 21/1/15.
14	3483	Hereford heifer...	14/1/15	10 c.c. blood <i>ex</i> 3475. Redwater and gallsickness blood	Reacted to redwater. From the 35th day <i>Anap. centrale</i> noted	Temperature reaction to redwater. <i>P. bigenimum</i> noted from the 21/1/15.
15	3477	Aberdeen Angus..	14/1/15	10 c.c. blood <i>ex</i> 3480. Redwater and gallsickness blood	Reacted to redwater. From the 28th day <i>Anap. centrale</i> noted	Temperature reaction to redwater. <i>P. bigenimum</i> in smears from the 23/1/15-27/1/15.
16	3484	Hereford heifer....	8/3/15	10 c.c. blood <i>ex</i> 3477. Redwater and gallsickness blood	Reacted to redwater. From the 28th day <i>Anap. centrale</i> noted	Temperature reaction to redwater. <i>P. bigenimum</i> noted on the 17/3/15.
17	3479	Aberdeen Angus...	8/3/15	10 c.c. blood <i>ex</i> 3483. Redwater and gallsickness blood	Reacted to redwater. From the 40th day <i>Anap. centrale</i> noted	Temperature reaction to redwater. <i>P. bigenimum</i> noted from the 17/3/15.
18	3478	Aberdeen Angus....	8/3/15	10 c.c. blood <i>ex</i> 3482. Redwater and gallsickness blood	Reacted to redwater. From the 38th day <i>Anap. centrale</i> noted	Temperature reaction to redwater. <i>P. bigenimum</i> noted from the 17/3/15.

Table No. 4.

No. of Expt.	No. of Animal in Expt.	Breed.	Date Inoculated.	Inoculated subcutaneously with.	Result.	Remarks.
1	2651	Hereford heifer (imported)	7/8/12	10 c.c. blood ex 1913.....	Reacted to redwater. From the 56th day <i>Anap. marginale</i> and <i>centrale</i> noted	Temperature reaction to redwater marked. <i>P. bigemina</i> noted.
2	3049	Friesland heifer (imported)	1/11/13	10 c.c. blood ex 2651. Redwater-gallsickness blood	Reacted to redwater. From the 43rd day <i>Anap. centrale</i> noted	Temperature reaction to redwater. <i>P. bigemina</i> noted from the 18/11/13-21/11/13.
3	3123	Aberdeen Angus (imported)	30/12/13	10 c.c. blood ex 3049. Redwater-gallsickness blood	Reacted to redwater. From the 33rd day <i>Anap. centrale</i> noted	Well marked temperature reaction to redwater from the 7th day. <i>P. bigemina</i> noted from the 6/1/14-9/1/14.
4	3124	Aberdeen Angus (imported)	30/12/13	10 c.c. blood ex 3049. Redwater-gallsickness blood	Reacted to redwater. From the 29th day <i>Anap. centrale</i> noted	Well marked temperature reaction from the 6th day. <i>P. bigemina</i> noted from the 6/1/14-8/1/14.
5	3125	Aberdeen Angus (imported)	30/12/13	10 c.c. blood ex 3049. Redwater-gallsickness blood	Reacted to redwater. From the 26th day <i>Anap. centrale</i> noted	Temperature reaction from the 6th day. <i>P. bigemina</i> noted on the 8/1/14 and 10/1/14.
6	3126	Aberdeen Angus (imported)	30/12/13	10 c.c. blood ex 3049. Redwater-gallsickness blood	Reacted to redwater. From the 22nd day <i>Anap. centrale</i> noted	Temperature reaction to redwater. <i>P. bigemina</i> noted from the 7/1/14-11/1/14.

Table No. 4 (continued).

No. of Expt.	No. of Animal in Expt.	Breed.	Date Inoculated.	Inoculated subcutaneously with.	Result.	Remarks.
7	3128	Aberdeen Angus (imported)	28/4/14	10 c.c. blood ex 3125. Redwater-gallsickness blood	From the 18th day <i>Anap. marginale</i> noted	No temperature reaction to redwater. No <i>P. bigemini</i> um noted in the smears. Died of anaplasmosis.
8	3279	South African bred heifer (Control)	28/4/14	10 c.c. blood ex 3125. Redwater-gallsickness blood	From the 19th day <i>Anap. marginale</i> noted	No temperature reaction to redwater. No <i>P. bigemini</i> um noted.
9	3286	South African bred heifer (Control)	28/4/14	10 c.c. blood ex 3125. Redwater-gallsickness blood	Reacted to redwater. From the 25th day <i>Anap. marginale</i> noted	Temperature reaction to redwater. <i>P. bigemini</i> um noted from the 12/5/14-15-5-14.
10	3272	South African bred calf (Control)	9/5/14	10 c.c. blood ex 3126. Redwater-gallsickness blood	From the 29th day <i>Anap. marginale</i> noted	Temperature reaction to redwater. From 19th day pathological blood change sequel of redwater.
11	3260	South African bred calf (Control)	9/5/14	10 c.c. blood ex 3049. Redwater-gallsickness blood	From the 29th day <i>Anap. marginale</i> noted	Slight temperature reaction to redwater. No <i>P. bigemini</i> um seen.
12	3306	South African bred calf (Control)	12/5/14	10 c.c. blood ex 3049. Redwater-gallsickness blood	Reacted to redwater. From the 26th day <i>Anap. marginale</i> noted	Temperature reaction to redwater. <i>P. bigemini</i> um noted on the 6th day.
13	3258	South African bred calf (Control)	12/5/14	10 c.c. blood ex 3126. Redwater-gallsickness blood	From the 29th day <i>Anap. marginale</i> noted. From the 3rd day pathological blood changes sequel of redwater noted	Temperature reaction to redwater. From the 9th day anisocytosis. No <i>P. bigemini</i> um noted till the 31st day.
14	3430	South African bred calf (Control)	4/9/14	10 c.c. blood ex 3049. Redwater and gallsickness blood	Reacted to redwater. From the 21st day <i>Anap. marginale</i> noted	Temperature reaction to redwater. <i>P. bigemini</i> um noted on the 18th day.



Genealogical Chart showing the mutability of Anaplasma Centrale.

The Cultivation of *Anaplasma marginale* in Vitro.

By

Dr. FRANK VEGLIA,

Veterinary Research Laboratories, Onderstepoort.

The Cultivation of *Anaplasma marginale* in Vitro.

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IN the course of some experiments undertaken with the object of separating *Anaplasma marginale* from similar intra-corpuscular bodies, and from other protozoa of a similar nature, I attempted the cultivation of anaplasma in vitro. The first attempts were already positive, and the results of these experiments form the subject of this paper. The culture media utilized were:—

- (1) Pure defibrinated blood, viz., blood from an animal infected with anaplasmosis.
- (2) Salt solution (sodium chloride) as used by Nuttal for the cultivation of *Babesia canis*.
- (3) Sodium citrate solution, as used by Carpano for the cultivation of *Nuttallia equi*.
- (4) Ordinary bouillon, as used by Miyajima for the cultivation of *Babesia bovis*.

These cultures are still being kept under observation, but so far the following results have been obtained:—

Carpano Medium.—The first culture was started with blood containing 8 per cent. of infected corpuscles and within 3 days the anaplasma reached 25 per cent. During the 40 following days the number of parasites varied between 6 per cent. as a minimum and 16 per cent. as a maximum.

Culture No. 3 was started with blood containing 7 per cent. of anaplasma, and in 20 days the infection had reached 20 per cent.; by the 40th day, 25 per cent. of the corpuscles were infected.

Culture No. 4 was commenced with blood containing 17 per cent. of infected red corpuscles, and within 21 days the infection gradually rose to 30 per cent.

In the fifth culture started with blood containing 1–2 per cent. infected corpuscles, the increase of the anaplasma oscillated between 4 per cent. and 8 per cent. for 21 days, and then suddenly rose to 20 per cent. within the next 3 days.

In the sixth culture, evidently a weak infection, since the animal itself only showed a mild reaction, starting with 0·5 per cent., the infection rose to 5 per cent. within 14 days.

In the tenth culture, the infection rose from 6 per cent. to 20 per cent. within 8 days.

Sodium Chloride.—In the culture with 0·6 per cent. sodium chloride, the experiments were equally successful, some cultures showing an increase from 6 per cent. to 20 per cent. of infected red corpuscles within 8 days.

Ordinary Bouillon.—Up to the present these cultures have only shown an increase to about half of the infection recorded in others, although in the tenth culture the infection rose from 6 per cent. to 13 per cent. within 8 days.

Pure Defibrinated Blood.—Blood when removed from an infected animal has proved to be successful medium for the cultivation of anaplasma.

In the third culture, starting with 16 per cent. infected red corpuscles, 24 per cent. was recorded in 4 days, whilst the same culture in Carpano medium only after 24 days had elapsed showed this number.

In the first culture, the development was slower in the defibrinated blood than in the Carpano medium, although the same maximum was reached.

In the sixth culture, in the Carpano medium the anaplasms developed better compared with that of defibrinated blood.

In the tenth culture with defibrinated blood, the anaplasms rose from 6 per cent. to 15 per cent. in 8 days, whilst in the Carpano medium 20 per cent. was recorded.

In the twelfth culture, both defibrinated blood and Carpano medium gave the same result in 5 days, when an increase from 3 per cent. to 11 per cent. was noted.

The various cultures referred to above were kept at 7° C., 15° C., and 26° C., but little difference was noted in the development of the anaplasms at these temperatures. It could be noted that with cultures kept at 7° C., the anaplasms developed rather more slowly, and the bacterial infection was not so strong as in the cultures kept at 15° C. and 26° C. Also in the case of the cultures kept at 7° C., haemolysis was also retarded by the lower temperature.

Cultures made with a small quantity of blood gave the best results.

The mixing of infected defibrinated blood, with clean defibrinated blood in equal parts, was followed by the development of the anaplasms. For instance, in a culture of the third series thus made the infection rose from 3 per cent. to 16 per cent. in 21 days.

Cultures of the fourth series increased from 7 per cent. to 12 per cent. under similar conditions in 26 days. The blood of an imported heifer, recently immunized against anaplasmosis, was also used as a culture medium and mixed with infected blood. The culture started with an 8 per cent. infection, and decreased to 2 per cent. during the 26 days of observation, whilst the infected blood itself in culture rose to 30 per cent. within the same period.

In another attempt infected blood of a second heifer was mixed with the blood of the above immune heifer, and the number of the anaplasms, starting from 0.5 per cent., rose to 2 per cent., and kept at about 1 per cent. during the following 21 days, whereas the infected blood not mixed with immune blood reached a 20 per cent. infection in the same time.

A sub-culture was attempted from a culture, when 11 days old, containing at the time 18 per cent. of infected red corpuscles. The blood utilized for the sub-culture was taken from a South African born animal.

The sub-culture was started with 3 per cent. infected red corpuscles, and it increased to 7 per cent. within 16 days.

Control cultures were also made with the blood of a normal calf not infected with anaplasmosis, and also with the blood of a sheep. During 20 days' observation no bodies could be noted resembling anaplasms.

When examining the cultures the precaution was taken of obtaining the blood from the various depths in the test tube. The infected corpuscles were counted out, and no difference was found in the blood taken from the top or bottom of the test tube.

The first parasites found in the cultures were the round forms, and when the culture became older they increased in size. In old cultures large forms were found of irregular, elongated, triangular, or quadrangular shape. Small anaplasms were also found, sometimes very numerous, and totalling 50 per cent. of all anaplasms present. They appeared to be succeeded by medium-sized anaplasms, which in turn were followed by the large anaplasms. The large forms of anaplasms by simple multiple division produced the smaller forms, and accordingly the larger parasites can be considered as schizontes and the smaller parasites as merozoites, and the process of multiplication would correspond to schizogony.

Forms with 5 and 8 merozoites were also found. Whilst anaplasms multiply in the culture an increase of the anaplasms in the blood of the cattle from which the culture was made was also noted, but only simple division of the anaplasms could be detected. It appears that when a culture is ageing the anaplasms of medium size are found in division. At a temperature of 26° C. this phenomenon was noted in cultures of 14 days' growth.

During all these observations no forms could be found resembling either piroplasms or the nuclei of erythroblast such as are frequently found in cases of anaemia. In cultures made with blood showing strong anaemia (particularly basophilia and polychromasia) the basophilia disappeared after 24 hours and the polychromasia disappeared within 3 days.

An attempt was made to see if Jolly's bodies present in the blood of a sheep would increase on culture media. It was found that their number decreased, and at the same time some of them when stained with Pappenheim showed a blue instead of a purple stain, thus indicating that they had undergone degeneration.

The contamination of the blood occasionally interferes with the development of the anaplasms. It was found, however, that in certain cultures, notwithstanding contamination, the anaplasms persisted.

The material used for the cultures was obtained both from animals at the beginning of the infection, when the number of the anaplasms was on the increase, and during the infection, when the number was on the decrease. All cultures made with such material were successful. Two of the cultures (Nos. 3 and 5) were made with blood infected with both anaplasmosis and babesiosis. The latter did not develop and the culture proved to contain anaplasms alone.

Conclusions :

(1) Anaplasms grow and multiply in the different artificial media indicated above.

(2) Defibrinated blood and Carpano media are most suitable for the cultivation of anaplasms.

(3) Basophile granulations and Jolly's bodies, which are noted in anaemic blood, behaved differently to anaplasms on cultivation, inasmuch as they did not increase in number and finally disappeared.

(4) Normal blood of cattle and other animals not infected with anaplasms did not develop any intracellular bodies resembling anaplasms when cultivated in the way indicated.

(5) In cultures made with pure anaplasms or anaplasms and babesia mixed, no forms could be traced which would suggest a transition of one to another.

(6) The defibrinated blood of a susceptible normal animal mixed with a culture appeared to be favourable for the development of the anaplasms, whilst the blood of an animal recovered from the recent attack of anaplasmosis appeared to inhibit the growth of the anaplasms.

(7) The cultivation of anaplasms by a sub-culture has so far succeeded in the one instance attempted.

(8) It appears that it is possible to grow anaplasms taken from an infected animal during the incubation stage of the disease.

(9) The increase of anaplasms in the cultures corresponds to the increase of the anaplasms in the living animal.

The oscillating decrease in the number of the anaplasms noted in an animal that is recovering has its simile in the cultures.

Aspergillosis in the Ostrich Chick.
(Synonyms : Yellow Liver, Chick Fever.)

BY
JAMES WALKER,
Veterinary Research Laboratories, Onderstepoort.

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INTRODUCTION.

AMONGST the class Aves a number of species such as the pigeon, turkey, pheasant, and especially the common fowl and others have been utilized for domestic purposes for long periods of time, and the diseases occurring amongst these have at various times been the subject of much investigation. In South Africa the history of ostrich farming dates back to not more than about sixty years, and it is only within recent times that this species formed the basis of a large industry. For these reasons a study of diseases affecting the ostrich chick has only lately demanded serious attention.

On referring to the literature in ostrich diseases in South Africa it will be found that the late Hon. Arthur Douglas, of Heatherton Towers, Cape Province, in his book entitled "Ostrich Farming in South Africa, 1881," devoted some chapters to an account of the diseases of the ostrich, and stated that, whereas in previous years 10 per cent. to 20 per cent. was the extent of losses, including accidents, amongst chicks, the mortality had by that time increased tremendously, so much so that on some farms every chick was swept off owing to a disease which even in those days was known as "Chick Fever" or "Yellow Liver." It appears that this disease was first reported from the Middelburg District and subsequently spread to the Colesberg, Cradock, and Albany Districts, Cape Province. It is important to note that this observer describes, amongst others, the following lesions :—

Small yellowish abscesses at the edge of the lobes of the liver and sometimes small ulcers on the tongue and entrance to the throat; the cause was ascribed to overfeeding. In Volume XX, June, 1912, of the *Cape Agricultural Journal*, Hutcheon described, under the term "Yellow Liver," a condition which affected chicks aged from one to six months, in which the whole of the appearances indicated anaemia, and, in his opinion, the cause was due to congenital functional debility, more particularly of the liver and digestive organs, and that its origin might be sought for in the feeding and management of parent birds, the aim of treatment being to limit the quantity of food.

INVESTIGATIONS.

In May, 1911, the writer received instructions from the Director of Veterinary Research to proceed to Grahamstown Veterinary Research Laboratory for duty and to make investigations in connection with diseases of the ostrich, and more particularly to make a study of chick fever, which was reported to occur on a number of farms and to be responsible

for the heavy mortality which many owners experienced. In fact, the losses on some farms were as high as 70 per cent. Considering the prices which were then paid for good birds, as high as £200-£300 for adults and £30 for three-month-old chicks, the prevention of chick fever was of economic importance.

On arrival, owners were invited to assist in the investigations, during the then coming 1911 and 1912 seasons, by forwarding sick or dead chicks to the Laboratory, so that an opportunity could be afforded of noting whether there were any pathological lesions characteristic of the disease and of securing material for transmission and cultural experiments. From the particulars furnished by a number of ostrich farmers it was found to be the general experience:

- (1) That although the usual methods of feeding and rearing had not been departed from, there was a variability in the number of cases which occurred during different seasons.
- (2) That the disease affected chicks from a few days up to three or four weeks old.
- (3) Cases were not confined to one particular hatching.

Various theories were brought forward as to the cause, as, for example:—

- 1st. Congenital weakness due to (a) in-breeding, (b) over-production.
- 2nd. Over-feeding on food such as rape, lucerne, etc.
- 3rd. Bacterial infection, more particularly of the alimentary tract.
- 5th. A pathological condition of the liver, hence the term "Yellow Liver."

As regards the symptoms there was found to be little or no difference in opinion, and they were described to be as follows:—Dullness, feeding indifferently, neck flexed, head carried close to body, loose and empty feel on pressure of abdomen, etc.

As to prevention the concensus of opinion was found to be that the rearing of chicks on clean ground was not effectual in preventing the appearance of the disease, but the majority of owners maintained that veld-hatched and veld-reared chicks were less affected than those hatched in incubators and hand reared.

As to Treatment.—Although various so-called remedies had been tried, there was apparently no known specific.

Mortality.—The percentage of recoveries of visibly affected chicks was practically nil. During the season of 1911-12 a number of post-mortems were made and some farms were visited on which a serious mortality amongst chicks was at the time occurring, with the result that a *Leucocytozoon* (*Leucocytozoon struthionis*) infection was noted (for a description and particulars of which see second report of the Director of Veterinary Research, 1912, page 384), and in October, 1912, the writer recorded the occurrence of aspergillosis in the ostrich in South Africa (Transactions of the Royal Society, Vol. III, Part 2, 1913), and at the latter end of the season the occurrence of aspergillosis in cases of so-called yellow liver or chick fever was observed.

As already stated, yellow liver was first recorded as far back as 1881. With the growth in importance of ostrich farming the demand for chicks from good feather strains and the rearing of ostriches increased considerably, so much so that artificial incubation was resorted to on a much larger scale than hitherto, on some farms as many as 1000 eggs being incubated in one season. Unfortunately with the increase in production, chick fever became more prevalent. The explanation of this will be seen later.

During the period between the end of 1911-12 and commencement of 1912-13 season some transmission experiments were made, with the result that *Aspergillus*, species *fumigatus* and *asper*, were found to be easily transmitted to the ostrich chick by means of the spores, the respiratory tract (lungs) being the chief seat of infection (for details of transmission experiments see Table No. 3, and a field experiment station was established at Table Farm, in the vicinity of Grahamstown, for the purpose of exposure experiments during the then coming season.

EXPOSURE EXPERIMENTS, TABLE FARM.

The object of this experiment was to expose chicks in a camp (lucerne) which was reputed to be very unhealthy, so much so that during previous seasons the mortality had been so great that the owner had decided to abandon the camp as a rearing ground, owing to the occurrence of the disease known as "Chick Fever."

For the purpose of this experiment it was necessary to have healthy chicks and these were obtained as follows:—

Several sets of breeding birds were camped off on an adjoining known healthy farm; the eggs were collected from these and incubated at the field experiment station in previously unused and new incubators. The chicks, when hatched, were kept at night for the first two or three days in the incubators and then boxed at night in paraffin boxes in which some clean sawdust was placed. During the day they were allowed to run in the reputed unhealthy lucerne camp. Unfortunately considerable difficulty was experienced in obtaining any number of eggs owing to the bad season—birds not laying well. No case of chick fever, however, occurred amongst the chicks in the experiment, but deaths resulted from other causes, such as eversion of the cloaca, taenia infection, accidents, etc. Table No. 1 gives details as to number of eggs incubated, number of chicks hatched, and date and particulars of death, etc.

Table No. 1.
EXPOSURE EXPERIMENTS.

No. of Chick.	Date noticed Sick.	Result.	Date of Death.	Age of Chick.	Symptoms Noted.	Cause of Death.	Cultures of Lung Tissue for Aspergillus Infection.	REMARKS.
224	20/11/12	Died	23/11/12	2 days	Weak and stunted at hatching	—	Negative	Chick had not been in exposure experiment.
225	20/11/12	"	24/11/12	3 days	"	—	"	"
230	—	"	3/12/12	A few days	None; found dead.....	Injury	"	"
236	2/12/12	"	4/12/12	"	Dull; not feeding.....	—	"	"
279	7/12/12	"	30/12/12	"	Weak and stunted at hatching	—	"	"
281	—	"	5/1/13	"	"	—	"	"
—	13/1/13	Recovered	—	3 months	Incoordination of movements.	—	—	"
—	13/1/13	"	—	"	"	—	—	"
—	14/1/13	"	—	"	"	—	—	"
284	—	Died	23/2/13	4 months	Collapse.....	Exposure	No cultures made	Chick exposed, owing to an accident, to heavy rain during night of 22/2/13.
285	—	"	23/2/13	"	"	"	"	"
319	—	"	23/2/13	12 days	"	"	"	"
288	27/2/13	"	28/2/13	3 months	Not feeding, lying (closed with petrol)	Enteritis	"	"
318	—	"	19/3/13	A few weeks	Eversion of cloaca.....	Eversion of cloaca	"	"
316	—	"	26/3/13	2 months	"	"	"	"
286	1/4/13	Destroyed	1/4/13	2½ months	"	"	"	"
317	—	Died	15/4/13	3 months	Eversion of cloaca.....	Eversion of cloaca Enteritis	"	Fracture of leg.
315	27/5/13	"	5/6/13	4 months	Dosed 27/5/13, off feed, lying, respirations increased	"	"	"
299	28/5/13	"	16/6/13	5 months	Dosed 27/5/13, off feed, lying, head thrown back to body	"	"	"
287	18/8/13	Destroyed	18/8/13	5½ months	—	—	"	Fracture of leg.

Table No. 1—continued.

INCUBATION OF EGGS FOR EXPOSURE EXPERIMENT.

No. of eggs incubated.	Date incubated.	No. of chicks hatched.
17	September, 1912	} Total 25.
43	November, 1912	
19	December, 1912	

Résumé of Table Farm Exposure Experiment.

Eggs incubated : Total, 79.

Chicks hatched, 25 ; died, 15 ; destroyed 2.

Summary of cause of deaths :—

Two : ages, 2½ and 5 months, respectively ; destroyed ; fracture of leg.

Three : ages, 12 days, 4 months, 4 months, respectively ; died from exposure to heavy rain during night.

Three : ages, 3, 4, and 5 months, respectively ; died from effects of dosing with petrol.

Three : ages, few weeks, 2 months, 3 months, respectively ; died from eversion of cloaca.

Four : ages, 2 days, 3 days, a few days, respectively ; died ; stunted and weak at hatching ; cultural experiment for aspergillosis negative.

One : age, a few days ; died from injuries.

One : age, a few days ; died ; stunted and weak at hatching ; cultural experiment for aspergillosis negative.

In order to obtain clean chicks for transmission and other experiments some incubators were erected at the Laboratory, Grahamstown. Considerable difficulty was experienced in securing a sufficiency of eggs, and those which were eventually obtained had to be conveyed some distance, with the result that the majority were found to be unsuitable for incubating. Out of a total of 121 incubated, only 18 hatched. As these were not sufficient to meet requirements, an endeavour was made to secure some common chicks from veld nests (the price of chicks reared by farmers for feathers being prohibitive), but, as it is the custom of owners to destroy veld-nest eggs, only a limited number of chicks were obtainable.

Since aspergillosis was found to occur, a careful search for lesions was made in making post-mortems on chicks suspected of being infected with or dead from chick fever, and cultures were made for the purpose of determining the presence or otherwise of this infection, and whenever an opportunity presented itself outbreaks of chick fever were investigated *in loco*, with the result that in a number of cases in which the owner ascribed the cause of death to yellow liver aspergillosis was found to be responsible.

It was not uncommon to find fungi other than *aspergillus* vegetating on media inoculated with material collected from the respiratory as well as the intestinal tract, the spores evidently having gained entrance with the inspired air or food, but since no pathological changes were caused by these, their presence, from a pathogenic point of view, is probably of

not much importance. A number of these were submitted to the Government Plant Pathologist and Mycologist for the Union Department of Agriculture, Pretoria, for identification and determined to be as follows :—

Oospora, *Sacharomyces*, *A. cladosporium*, *Mucor A*, *Mucor B*, *Sporotricheum*, *Rhizopus nigricans*, *Penicillium glaucum*, *A Torula*, and Fungi with a sterile mycelium.

Several species of aspergillus were found to occur in the chick. These were also submitted and identified, viz. :—

Aspergillus asper, *Aspergillus albus*. *Aspergillus* comes nearest to *Asp. niger*, *Aspergillus* comes nearest to *Asp. clavatus*.

The commoner and more pathogenic was found to be *Aspergillus fumigatus*.

During the 1912-13 season some further transmission experiments were carried out. (For details of transmission experiments, see Table No. 3.)

The results of post-mortem examinations showed that the respiratory tract (lungs and thoracic air sacs) was the common seat of infection, and it was necessary to ascertain under what conditions aspergillosis was contracted naturally. It was noted to be the custom on many farms to put young chicks at night in boxes containing straw, and it occurred to the writer that the straw might be the source of infection.

In 1913 an outbreak of chick fever was investigated in the Alexandria District, Cape Province. From the history it was difficult to account for the outbreak, as the chicks were taken from the incubators about the 3rd day after hatching and kept on clean ground and in new sheds. Cultures were made from the straw in the boxes used and found to be infected. It was then decided to note the effects of boxing chicks in boxes containing straw from the same stack as that being used on the farm, with the result that aspergillosis was contracted by the chicks. This experiment was repeated later. (For details, see Table No. 2.) On the 16th October, 1913, Mr. Robertson, M.R.C.V.S., Assistant Director of Veterinary Research, arrived at Grahamstown and took over charge of the Laboratory, and on my return to headquarters I was instructed to continue investigations in connection with ostrich chick diseases. The general outline of experiments was drafted by the Director of Veterinary Research, and was as follows :—

1. To repeat experiments with infected straw.
2. To repeat transmission experiments.
3. To make a bacteriological examination of all ostrich eggs which were incubated, but had not hatched, in the Laboratory incubators.
4. A post-mortem examination of all dead ostrich chicks hatched at the Laboratory.

Experiments were also undertaken to ascertain :—

5. The effects of infecting the air chamber of incubating eggs with *Aspergillus fumigatus* spores.
6. Whether the incubators were infected.
7. Whether eggs the shells of which were smeared with a culture of *Aspergillus fumigatus* would become infected.
(Fowls eggs were used for this experiment.)

Some clean eggs were also placed in the same incubator for the purpose of noting whether they would become infected.

As the faeces of an ostrich appeared to be a probable means of dissemination of aspergillus, the following experiments were made to ascertain whether the spores vegetated after passing through the intestinal canal:—

8. Ostriches were dosed with spores of *Aspergillus fumigatus* and cultures made from the faeces.
9. Cultures were made from the faeces of an ostrich being fed on lucerne.
10. Cultures were made from lucerne (and soil) collected from a field the lucerne in which was being used for feeding chicks.
11. Experiments were also carried out to note effectiveness of boiling water and steam for sterilising cultures of fungi, such as aspergillus.

Dealing separately with the above experiments:—

(1) *Repeated Experiments with infected Straw.* (For details of this and former experiments, see Table No. 2.)

Table No. 2.
INFECTED STRAW EXPERIMENTS.

No. of Experiment.	No. of Chick.	Age of Chick when put in Experiment.	Date of Experiment.	Method of Boxing.	Result.	Date of Death.	Symptoms.	Pathological-Anatomical Appearances.	Result of Culture Experiment for Aspergillus Infection.	Cause of Death.
1	361	8 days	22/9/13	The method was similar to that carried out on many ostrich farms, viz.:—The chicks were put at night in empty paraffin boxes containing straw and the top of the box partly covered with a sack. The number of chicks put in a box depended on the size of the birds, four being the average number for chicks to about three weeks old. (The straw used for this experiment was obtained from a farm in the Cape Province. <i>Aspergillus fumigatus</i> was found to be the commoner species in this straw.)	Died	28/9/13	Dull, not feeding, respiration accelerated, temperature irregular	Tubercles very rare in lung	Aspergillus growth (fumigatus)	Aspergillosis (pneumomycosis)
2	362	7 days	22/9/13		"	26/9/13	From the 24/9/13 temperature sub-normal	Slight hyperaemia pulmonum	"	"
3	363	6 days	22/9/13		"	25/9/13	Dull, not feeding, respiration accelerated, temperature irregular	"	"	"
4	322	10 days	20/11/13		"	29/11/13	From the 24/11/13 noted dull and unthrifty looking, head carried low, temperature irregular from 23/11/13; respiration slightly increased from 27/11/13, average 22 per minute	Pleuritis fibrinosa. Tubercles rare on wall of thoracic air-sacs and pleura	"	"
5	314	10 days	20/11/13		Remained healthy	—	—	—	—	—
6	330	8 days	29/11/13	The method was similar to that carried out on many ostrich farms, viz.:—The chicks were put at night in empty paraffin boxes containing straw and the top of the box partly covered with a sack. The number of chicks put in a box depended on the size of the birds, four being the average number for chicks to about three weeks old. (The straw used for this experiment was obtained from a farm in the Cape Province. <i>Aspergillus fumigatus</i> was found to be the commoner species in this straw.)	Died	3/12/13	Temperature irregular from 1/12/13; from 2/12/13 dull and unthrifty looking	Slight hyperaemia pulmonum	Aspergillus growth (fumigatus)	Aspergillosis (pneumomycosis)
7	331	3 days	29/11/13		Remained healthy	—	—	—	—	—
8	344	5 days	1/1/14		Died	9/1/14	From 5/1/14 noted dull and unthrifty looking, not feeding well, head carried low; chick frequently opens beak, taking a deep inspiration	Slight hyperaemia pulmonum	Aspergillus growth (fumigatus)	Aspergillosis (pneumomycosis)
9	345	4 days	1/1/14		"	18/1/14	From 5/1/14 dull and unthrifty looking, not feeding well; morning temperature sub-normal from 8/1/14, average 95° F.; head carried low	Tubercles rare in right lung	"	"

Table No. 2—(continued).

No. of Experiment.	No. of Chick.	Age of Chick when put in Experiment.	Date put in Experiment.	Method of Boxing.	Result.	Date of Death.	Symptoms.	Pathological-Anatomical Appearances.	Result of Culture Experiment for Aspergillus Infection.	Cause of Death.
1	309	30 days	5/12/13	Boxed at night in box containing straw previously sterilized and then infected with <i>Aspergillus fumigatus</i> spores recovered from straw sent in from a farm in the Cape Province.	Died	12/12/13	From 8/12/13 respirations increased, beak frequently opened	Tubercles frequent in lungs and on walls of air sacs	Aspergillus growth (<i>fumigatus</i>)	Aspergillosis (pneumomycosis)
2	296	31 days	5/12/13		"	12/12/13	From 10/12/13 respirations increased, looking dull and unthrifty, not feeding well	Tubercles frequent in lungs and rare on walls of air sacs	"	"
3	286	45 days	12/12/13	Boxed at night in straw containing Aspergillus species asper. Aspergillus recovered from straw sent in from a farm in the Cape Province.	Remained healthy	—	—	—	—	—
4	318	32 days	12/12/13		"	—	—	—	—	—
5	342	9 days	6/1/14		Died	11/1/14	From 8/1/14 temperature irregular; morning, sub-normal temperature; not feeding well; respirations visibly increased and beak opening frequently from 10/1/14	Hyperaemia pulmonum; tubercles frequent in lungs and on walls of air sacs	Aspergillus growth (<i>asper</i>)	Aspergillosis (pneumomycosis)
6	343	9 days	6/1/14	Boxed at night in straw containing Aspergillus species asper. Aspergillus recovered from straw sent in from a farm in the Cape Province.	"	13/1/14	From 9/1/14 morning temperature sub-normal	"	"	"

Résumé.

Aspergillosis was contracted by chicks, ages from 3 to 10 days, in seven out of nine experiments, boxed at night in boxes containing straw naturally infected with *Aspergillus fungi* (*fumigatus* being the commoner species present in the straw), death resulting in from 3-17 days.

Chicks, ages 30 days and 31 days respectively, boxed at night in boxes containing straw previously sterilized and infected artificially with *Aspergillus fumigatus* spores, contracted aspergillosis. Death resulted in each instance in seven days.

Chicks, ages 9 days respectively, boxed at night in boxes containing straw previously sterilized and infected artificially with *Aspergillus*, species *asper*, in two out of four experiments contracted aspergillosis, death resulting in from 5-7 days. The remaining two chicks, ages 32 and 45 days, respectively, did not contract aspergillosis.

(2) *Transmission Experiments.*

(For details see Table No. 3.)

Table No. 3.
TRANSMISSION EXPERIMENTS.

No. of Experiment.	No. of Chick.	Age of Chick.	Date of Experiment.	EXPERIMENT.	Result of Experiment.	Date of Death.	Cultural Experiments for Aspergillus Infection.	Cause of Death.	REMARKS.
1	108	A few days	5/8/12	Inhalation—Spores of <i>Aspergillus fumigatus</i> blown into trachea by means of a pipette	Died	7/8/12	Aspergillus infection	Aspergillosis	
2	107	7 days	5/8/12		"	8/8/12	"	"	"
3	294	6 days	10/11/13		"	14/11/13	"	"	"
4	290	6 days	10/11/13		"	15/11/13	"	"	"
5	293	17 days	20/11/13		"	28/11/13	"	"	"
6	328	12 days	29/11/13		"	2/12/13	"	"	"
1	136	A few days	3/9/12	Inhalation—Spores of <i>Aspergillus fumigatus</i> blown into trachea by means of a pipette	Died	5/9/12	Aspergillus infection	Aspergillosis	
2	329	14 days	1/12/13		"	—	"	"	"
3	313	31 days	6/12/13		"	—	"	"	"
4	346	6 days	3/1/14		"	—	"	"	"
1	104	8 months	31/5/12	Intravenous inoculation, 2 c.c. of a bouillon lactose glycerine culture	Died	7/6/12	Aspergillus infection	Aspergillosis	
2	101	9 months	5/7/12		"	9/7/12	"	"	
1	229	3 weeks	15/12/12	Dosed with <i>Aspergillus fumigatus</i> spores in gelatine capsule	Died	20/12/12	Aspergillus infection	Aspergillosis	
2	227	"	15/12/12		"	20/12/12	"	"	
3	232	"	15/12/12		"	20/12/12	"	"	
4	231	"	15/12/12		"	23/12/12	Negative for asper.	"	
5	358	6 days	20/9/13		"	29/9/13	Aspergillus infection	Aspergillosis	
6	359	"	20/9/13		"	1/10/13	"	"	
7	360	"	20/9/13		"	8/10/13	"	"	
8	361	"	10/11/13		"	20/11/13	"	"	
9	292	8 days	10/11/13		"	—	"	"	Remained healthy.
10	331	41 days	6/1/14		"	—	"	"	"
11	40	Adult	15/1/14		"	—	"	"	"

*Résumé of Transmission Experiments.**(1) Inoculation Experiments.*

(a) Spores of *Aspergillus fumigatus*, blown into the trachea of young chicks (age from 5-17 days) by means of a pipette, caused death in from 2 to 8 days, the lesions being confined to the respiratory tract (lungs and air sacs).

(b) Spores of *Aspergillus asper*, blown into the trachea of young chicks (age from a few to 31 days) by means of a pipette, caused death in from 1-10 days, the lesions being confined to the respiratory tract (lungs and air sacs).

(2) Intravenous injection of a bouillon lactose glycerine culture, 2-5 c.c., resulted in death in from 2-7 days, the lesions being pronounced in the lungs and liver.

(3) (a) The dosing of young chicks with spores of *Aspergillus fumigatus* in a gelatine capsule resulted in death in from 5 to 18 days in seven out of nine experiments, the lesions being located in the respiratory tract (lungs or thoracic air sacs).

(b) The dosing of older birds (a 41 days old and an adult) did not cause death, both remaining apparently healthy.

(3) Bacteriological Examination of Contents of Ostrich Eggs.

(For details see Table No. 4).

Table No. 4.
EXAMINATION OF EGGS.

Egg No.	Date Incubated.	Date Culture Made.	Condition of Contents of Egg.	Media inoculated with Portion of Contents and incubated Aerobically.	Media inoculated with Portion of Contents and incubated Anaerobically.	Microscopical Examination of Smears of Growth.	Egg incubated in Incubator No.	REMARKS.
1	7/10/13	25/10/13	Contents decomposed; on shaking, portion of contents escape through unbroken shell	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods)	2	
2	29/9/13	25/10/13	Slight putrefactive odour; contents thin liquid	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods) Gram. positive organism (cocci and cocco-bacillary forms)	2	
3	27/9/13	27/10/13	Contents decomposed, putrefactive odour; contents escaping through unbroken shell	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods) Gram. positive organism (cocci, cocco-bacillus, and short rods)	2	
4	23/9/13	29/10/13	Unfertilized, no odour.....	No growth.....	No growth.....	Negative.....	2	
5	22/9/13	29/10/13	Unfertilized, no odour.....	No growth.....	No growth.....	Negative.....	2	
6	23/9/13	3/11/13	Slight putrefactive odour.....	Growth.....	Growth.....	Gram. negative organism (cocci, cocco-bacillus, and rods) Gram. positive cocci, resembling <i>Staphylococcus pyogenes aureus</i>	2	
7	23/9/13	3/11/13	Slight putrefactive odour, unfertile	Growth.....	Growth.....	Gram. positive cocci, resembling <i>Staphylococcus pyogenes aureus</i>	1	
8	23/9/13	3/11/13	Slight putrefactive odour, unfertile	Growth.....	Growth.....	Gram. negative organism (cocci and cocco-bacillus) Gram. positive organism (cocco-bacillus)	1	
9	27/9/13	3/11/13	Slight putrefactive odour, unfertile	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods) Gram. positive organism (cocco-bacillus and rods)	2	
10	29/9/13	8/11/13	No putrefactive odour, unfertile.	No growth.....	No growth.....	Negative.....	2	

Table No. 4—(continued).

Egg No.	Date Incubated.	Date Culture Made.	Condition of Contents of Egg.	Media inoculated with Portion of Contents and incubated Aerobically.	Media inoculated with Portion of Contents and incubated Anaerobically.	Microscopical Examination of Smears of Growth.	Egg incubated in Incubator No.	REMARKS.
11	29/9/13	8/11/13	Contents decomposed, putrefactive odour	Growth.	Growth.	Gram. negative organism (cocco-bacillus and rods)	2	
12	5/11/13	14/11/13	Contents decomposed, putrefactive odour	Growth.	Growth.	Gram. negative organism (cocco-bacillus and rods)	1	
13	6/11/13	14/11/13	Slight putrefactive odour, unfertile	Growth.	Growth.	Gram. negative organism (cocco-bacillus and rods)	1	
14	5/11/13	14/11/13	No putrefactive odour, unfertile.	No growth.	No growth.	Negative.	1	
15	5/11/13	14/11/13	Contents decomposed, putrefactive odour	Growth.	Growth.	Gram. negative organism (cocco-bacillus and rods)	1	
16	5/11/13	14/11/13	Contents decomposed, putrefactive odour	Growth.	Growth.	Gram. negative organism (cocco-bacillus and rods)	1	
17	5/11/13	14/11/13	Slight putrefactive odour, unfertile	Growth.	Growth.	Gram. negative organism (cocco-bacillus and rods)	1	
18	5/11/13	14/11/13	Slight putrefactive odour, unfertile	Growth.	Growth.	Gram. negative organism (cocco-bacillus and rods)	1	
19	5/11/13	14/11/13	Unfertile, no odour.	No growth.	No growth.	Negative.	1	
20	5/11/13	14/11/13	Slight putrefactive odour.	Growth.	Growth.	Gram. negative organism (cocco-bacillus and rods)	1	
21	5/11/13	14/11/13	Slight putrefactive odour.	Growth.	Growth.	Gram. negative organism (cocco-bacillus and rods)	1	
22	5/11/13	14/11/13	Slight putrefactive odour.	Growth.	Growth.	Gram. negative organism (cocco-bacillus and rods)	1	
23	5/11/13	14/11/13	Slight putrefactive odour.	Growth.	Growth.	Gram. negative organism (cocco-bacillus and rods)	1	
24	5/11/13	17/11/13	Putrefactive odour (contents decomposed)	Growth.	Growth.	Gram. negative organism (cocco-bacillus and rods)	1	

Table No. 4—(continued).

Egg No.	Date Incubated.	Date Culture Made.	Condition of Contents of Egg.	Media inoculated with Portion of Contents and incubated Aerobically.	Media inoculated with Portion of Contents and incubated Anaerobically.	Microscopical Examination of Smears of Growth.	Egg incubated in Incubator No.	REMARKS.
25	5/11/13	17/11/13	Putrefactive odour (contents decomposed)	Growth.....	Growth.....	Gram. negative organism (coccobacillus and rods)	1	
26	7/11/13	17/11/13	Putrefactive odour (contents decomposed)	Growth.....	Growth.....	Gram. negative organism (coccobacillus and rods)	2	
27	5/11/13	18/11/13	Putrefactive odour, decomposed; contents escaping through unbroken shell	Growth.....	Growth.....	Gram. positive organism (coccobacillus and short rods)	1	
28	5/11/13	19/11/13	Putrefactive odour, decomposed; contents escaping through unbroken shell	Growth.....	Growth.....	Gram. negative organism (coccobacillus and rods) Gram. positive organism (coccobacillus and rods)	1	
29	16/10/13	20/11/13	Putrefactive odour, decomposed; fungus growth in air chamber; contents decomposed	Growth.....	Growth.....	Gram. negative organism (coccobacillus and rods) Gram. positive organism (coccobacillus and rods)	1	
30	5/11/13	23/11/13	Putrefactive odour, decomposed; contents escaping through shell wall	Growth.....	Growth.....	Gram. negative organism (coccobacillus and rods) Gram. positive organism (coccobacillus and rods)	3	
31	5/11/13	24/11/13	Putrefactive odour, decomposed; contents escaping through shell wall	Growth.....	Growth.....	Gram. negative organism (coccobacillus and rods) Gram. positive organism (coccobacillus and rods)	1	
32	5/11/13	24/11/13	Putrefactive odour; contents escaping through unbroken shell	Growth.....	Growth.....	Gram. negative organism (coccobacillus and rods) Gram. positive organism (coccobacillus and rods)	1	

Table No. 4—(continued).

Egg No.	Date Incubated.	Date Culture Made.	Condition of Contents of Egg.	Media inoculated with Portion of Contents and incubated Aerobically.	Media inoculated with Portion of Contents and incubated Anaerobically.	Microscopical Examination Smears of Growth.	Egg incubated in Incubator No.	REMARKS.
33	14/11/13	25/11/13	Putrefactive odour, contents decomposed	Growth.....	Growth.....	Gram, negative organism (coccobacillus and rods) Gram, positive organism (coccobacillus and rods)	1	
34	12/11/13	25/11/13	Putrefactive odour.....	Growth.....	Growth.....	Gram, negative organism (coccobacillus and rods) Gram, positive organism (coccobacillus and rods)	1	
35	12/11/13	25/11/13	No putrefactive odour.....	No growth.....	No growth.....	Negative.....	2	
36	15/11/13	27/11/13	Putrefactive odour; contents decomposed	Growth.....	Growth.....	Gram, negative organism (coccobacillus and rods) Gram, positive organism (coccobacillus and rods)	1	
37	14/11/13	27/11/13	Putrefactive odour; contents decomposed and escaping through shell wall	Growth.....	Growth.....	Gram, negative organism (coccobacillus and rods) Gram, positive organism (coccobacillus and rods)	1	
38	16/10/13	27/11/13	Putrefactive odour; contents decomposed	No cultures made	—	—	2	Shell broken previous to arrival at Laboratory.
39	16/10/13	27/11/13	Putrefactive odour; contents decomposed	No cultures made	—	—	2	"
40	16/10/13	27/11/13	Putrefactive odour; contents decomposed	Growth.....	Growth.....	Gram, negative organism (coccobacillus and rods) Gram, positive organism (coccobacillus and rods)	2	"
41	5/11/13	28/11/13	Putrefactive odour; contents decomposed and escaping through shell wall	Growth.....	Growth.....	Gram, negative organism (coccobacillus and rods) Gram, positive organism (coccobacillus and rods)	1	

Table No. 4—(continued).

Egg No.	Date Incubated.	Date Culture Made.	Condition of Contents of Egg.	Media inoculated with Portion of Contents and incubated Aerobically.	Media inoculated with Portion of Contents and incubated Anaerobically.	Microscopical Examination of Smears of Growth.	Egg incubated in Incubator No.	REMARKS.
42	5/11/13	1/12/13	Putrefactive odour; contents decomposed and escaping through shell wall	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods) Gram. positive organism (cocco-bacillus and rods)	1	
43	14/11/13	2/12/13	Putrefactive odour; contents decomposed and escaping through shell wall	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods) Gram. positive organism (cocci, cocco-bacillus, and short rods)	1	
44	5/11/13	2/12/13	Putrefactive odour; contents decomposed	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods)	1	
45	5/11/13	2/12/13	Slight putrefactive odour.....	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods)	1	
46	5/11/13	3/12/13	Putrefactive odour; contents decomposed (<i>Aspergillus fumigatus</i> growth in air chamber)	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods) <i>Aspergillus fumigatus</i> aerobic	1	
47	5/11/13	3/12/13	Putrefactive odour; contents decomposed (<i>Aspergillus fumigatus</i> growth in air chamber)	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods) <i>Aspergillus fumigatus</i> aerobic	1	
48	18/11/13	3/12/13	Putrefactive odour; contents decomposed and escaping through shell wall (<i>Aspergillus fumigatus</i> growth in air chamber)	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods) Gram. positive organism (cocco-bacillus and rods) <i>Aspergillus fumigatus</i> aerobic	1	
49	14/11/13	3/12/13	Putrefactive odour; contents decomposed and escaping through shell wall	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods) Various gram. positive organisms	1	
50	14/11/13	3/12/13	Putrefactive odour; contents decomposed and escaping through shell wall	Growth.....	Growth.....	Various gram. negative and positive organisms	1	
51	14/11/13	5/12/13	Putrefactive odour; contents decomposed and escaping through shell wall	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods) Various gram. positive organisms	1	

Table No. 4—(continued).

Egg No.	Date Incubated.	Date Culture Made.	Condition of Contents of Egg.	Media inoculated with Portion of Contents and incubated Aerobically.	Media inoculated with Portion of Contents and incubated Anaerobically.	Microscopical Examination of Smears of Growth.	Egg incubated in Incubator No.	REMARKS.
52	14/11/13	5/12/13	Putrefactive odour; contents decomposed and escaping through shell wall	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods) Gram. positive organism (cocci, cocco-bacillus, and rods)	1	
53	14/11/13	6/12/13	Putrefactive odour; contents decomposed and escaping through shell wall	Growth.....	Growth.....	Gram. negative organism (cocci and cocco-bacilli) Various gram. positive organisms	1	
54	14/11/13	8/12/13	Slight putrefactive odour.....	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods) Gram. positive organism (cocci and cocco-bacilli)	1	
55	1/12/13	8/12/13	Slight putrefactive odour, unfertile	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods)	1	
56	5/11/13	9/12/13	Slight putrefactive odour; contents partly decomposed	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods) Gram. positive organism (cocco-bacillus and rods)	1	
57	5/11/13	9/12/13	Putrefactive odour; dead chick.	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods) Gram. positive organism (cocci, cocco-bacillus, and rods)	1	
58	12/11/13	9/12/13	Putrefactive odour; fungus growth on membrane	Growth.....	Growth.....	Gram. negative cocci and cocco-bacilli Various gram. positive organisms	1	
59	12/11/13	9/12/13	Putrefactive odour; contents decomposed; fungus growth on membrane	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods) Gram. positive organism (cocco-bacillus and rods)	1	
60	5/11/13	9/12/13	Putrefactive odour; contents decomposed	Growth.....	Growth.....	Gram. negative organism (cocco-bacillus and rods) Various gram. positive organisms	1	

Table No. 4—(continued).

Egg No.	Date Incubated.	Date Culture Made.	Condition of Contents of Egg.	Media inoculated with Portion of Contents and incubated Aerobically.	Media inoculated with Portion of Contents and incubated Anaerobically.	Microscopical Examination Smears of Growth.	Egg incubated in Incubator No.	REMARKS.
61	14/11/13	9/12/13	No putrefactive odour, unfertile.	No growth.....	No growth.....	Negative.....	1	
62	12/11/13	9/12/13	No putrefactive odour, unfertile.	No growth.....	No growth.....	Negative.....	2	
63	12/11/13	9/12/13	Slight putrefactive odour; contents decomposed	Growth.....	Growth.....	Gram, negative organism (cocco-bacillus and rods)	1	
64	5/11/13	9/12/13	No putrefactive odour.....	No growth.....	No growth.....	Negative.....	1	
65	18/11/13	12/12/13	Putrefactive odour; contents decomposed and escaping through shell wall	Growth.....	Growth.....	Gram, negative organism (cocco-bacillus and rods) Various gram, positive organisms	1	
66	18/11/13	12/12/13	Putrefactive odour; contents decomposed	Growth.....	Growth.....	Gram, negative organism (cocco-bacillus and rods)	1	
67	18/11/13	12/12/13	Putrefactive odour; contents decomposed and escaping through shell wall	Growth.....	Growth.....	Gram, negative organism (cocco-bacillus and rods) Gram, positive organism (coccus, cocco-bacillus, and rods)	1	
68	14/11/13	12/12/13	Putrefactive odour; contents decomposed and escaping through shell wall	Growth.....	Growth.....	Gram, negative organism (cocco-bacillus and rods)	1	
69	12/11/13	15/12/13	Putrefactive odour; contents decomposed and escaping through shell wall	Growth.....	Growth.....	Gram, negative organism (coccus, cocco-bacillus, and rods) Various gram, positive organisms	2	
70	18/11/13	17/12/13	Putrefactive odour; contents decomposed	Growth.....	Growth.....	Gram, negative organism (cocco-bacillus and rods) Gram, positive organism	2	
71	12/11/13	18/12/13	Putrefactive odour; contents decomposed; dead chick	Growth.....	Growth.....	Gram, negative organism (cocco-bacillus and rods)	2	
72	12/11/13	18/12/13	No putrefactive odour.....	No growth.....	No growth.....	Negative.....	—	
73	21/11/13	18/12/13	Slight putrefactive odour.....	Growth.....	Growth.....	Gram, negative organism (cocco-bacillus and rods) Gram, positive organism (cocco-bacillus and rods)	2	

Table No. 4—(continued).

Egg No.	Date Incubated.	Date Culture Made.	Condition of Contents of Egg.	Media inoculated with Portion of Contents and incubated Aerobically.	Media inoculated with Portion of Contents and incubated Anaerobically.	Microscopical Examination of Smears of Growth.	Egg incubated in Incubator No.	REMARKS.
74	18/11/13	18/12/13	Slight putrefactive odour.....	Growth.....	Growth.....	Gram. negative organism (coccobacillus and rods)	2	
75	12/11/13	18/12/13	Slight putrefactive odour.....	Growth.....	Growth.....	Gram. negative organism.....	2	
76	25/11/13	18/12/13	No putrefactive odour; dead chick	Negative.....	Negative.....	Negative.....	2	
77	25/11/13	18/12/13	No putrefactive odour; dead chick	Negative.....	Negative.....	Negative.....	2	
78	12/11/13	18/12/13	Slight putrefactive odour.....	Growth.....	Growth.....	Gram. negative organism (coccobacillus and rods) Gram. positive organism (coccobacillus)	2	
79	25/11/13	18/12/13	No putrefactive odour; dead chick	Negative.....	Negative.....	Negative.....	2	
80	25/11/13	20/12/13	Slight putrefactive odour.....	—	—	—	1	
81	1/12/13	31/12/13	No putrefactive odour.....	No cultures made	—	—	1	
82	4/12/13	13/1/14	Putrefactive odour.....	No cultures made	—	—	2	Shell broken when handed in for examination.
83	7/12/13	18/1/14	Putrefactive odour.....	No cultures made	—	—	2	"
84	7/12/13	13/1/14	Slight putrefactive odour.....	No cultures made	—	—	2	"
85	21/12/13	19/1/14	Putrefactive odour.....	—	—	Gram. negative organism (coccobacillus and rods) Gram. positive organism (coccobacillus and rods)	1	
86	21/12/13	20/1/14	No putrefactive odour.....	No growth.....	No growth.....	Negative.....	1	
87	10/1/14	3/2/14	Putrefactive odour; contents decomposed and escaping through unbroken shell	Growth.....	Growth.....	Gram. negative organism (coccobacillus and rods) Various gram. negative sporulating bacilli	2	

*Bacteriological Examination of Eggs.**Résumé.*

In all cases in which the contents *were decomposed* a bacterial infection was found to exist. In those cases in which the contents had no putrefactive odour no bacterial infection existed.

Aspergillus fumigatus was found lodged in the air chamber of some eggs, and cultures made from the inner membrane of the shells of others resulted in the growth of a fungus with a sterile mycelium.

The contents of *seventy-two eggs* had a putrefactive odour, which was more marked in some than in others, and were in various stages of decomposition, the contents of *twenty-two* being of a thinner consistency than normal, uniformly coloured yellowish throughout and escaping through the unbroken shell; in others the contents were blackish.

In *fifteen instances* no putrefactive odour was noted. Cultures were made aerobically on potato and acrobically and anaerobically on agar media from the contents of sixty-seven eggs which had a putrefactive odour. Cultures were made aerobically and anaerobically from fourteen eggs, the contents of which had no putrefactive odour.

Smears were made from the growth which resulted from the putrefactive contents, with the result that the following organism was found to be the more constantly present. Fig 6.

Colonies of the above were isolated and found to have the following characteristics, viz. :—

- (a) Form and size varies, sometimes occurring in form of a coccus, a cocco-bacillus, rods, and spiral threads.
 - (b) Mobility, actively motile.
 - (c) No spore formation.
 - (d) Gram negative.
 - (e) Size varies from 1-4 μ long and from .4- .7 μ thick.
 - (f) Grows on agar, potato, and gelatine aerobically and anaerobically at room temperature.
 - (g) *Gelatine* is rapidly liquefied.
- Agar*.—Colonies are thin, transparent, moist, extending over the whole surface.
- Potato*.—Yellowish-white growth, turning to brown.
- Milk*.—Coagulated and casein dissolved.
- Indol is produced in peptone medium.

Small Animal Inoculation.

Non-pathogenic for *guinea pigs and rabbits* if injected in small quantities subcutaneously.

The general characters of the above organism correspond with the common type of the *Proteus* group, viz., *Proteus vulgaris*.

A gramme positive organism occurring in the form of a coccus, a cocco-bacillus or short rods, was frequently noted (see Fig. 7.)

(4) *Post-mortem Examination and Cultures from Dead Chicks hatched at the Laboratory.*

(For details see Table No. 5.)

Table No. 5.

DETAILS OF CHICKS DEAD AT LABORATORY.

No. of Chick.	Date Hatched.	Age of Chick.	Symptoms.	Interim.	Pathological Anatomical Examination.	Result of Culture Experiments for Aspergillus Injection.	Cause of Death.	Date Egg Incubated.	No. of Incubator.	REMARKS.
270	9/10/13	18 days	Head carried low, noticed sick three days, dull, off feed, constipated; stunted and weak at time of hatching	6 hours	Hyperaemia pulmonum. Yellowish caseous-like nodule left lung	Aspergillus growth from nodule	Aspergillosis	30/8/13	1	
274	11/10/13	17 days	Stunted from time of hatching; weight at time of death, $\frac{3}{4}$ lb.	3 hours	Nil.....	Negative	—	30/8/13	1	
243	29/8/13	61 days	Stunted in growth.....	2 hours	Nil.....	Negative	—	20/7/13	3	
278	21/10/13	8 days	Morning of 29th respirations accelerated (44); head carried low, neck flexed	12 hours	Hyperaemia pulmonum	Negative	—	9/9/13	3	
283	29/10/13	1 day	Weak.....	12 hours	Hyperaemia pulmonum. Haemothorax and extravasation of blood in subcutaneous tissues of back	No cultures made	Injured at time of hatching	16/9/13	3	Chick well developed; shell broken to assist in hatching of chick.
288	30/10/13	1 day	Weak.....	12 hours	Hyperaemia pulmonum. Contents yolk sac decomposed	No cultures made	—	16/9/13	3	Chick well developed; shell broken to assist in hatching of chick.
289	29/10/13	36 hours	Weak.....	12 hours	Hyperaemia pulmonum and oedema	No cultures made	—	16/9/13	3	Chick well developed; shell broken to assist chick in hatching; head of chick lying at end of egg opposite air chamber.
290	30/10/13	1 day	Weak.....	12 hours	Hyperaemia pulmonum	No cultures made	—	16/9/13	3	Chick well developed; shell broken to assist chick to hatch; head of chick lying away from air chamber.

Table No. 5—(continued).

No. of Chick.	Date Hatched.	Date Died.	Age of Chick.	Symptoms.	Interim.	Pathological Anatomical Examination.	Result of Culture Experiments for Aspergillus Infection.	Cause of Death.	Date Egg Incubated.	No. of Incubator.	REMARKS.
302	Dead in shell	3/11/13	—	Weak.....	—	Hyperaemia pulmonum	No cultures made	—	23/9/13	1	Dead in shell; well developed; head of chick lying away from air chamber. Egg broken 3/11/13.
303	3/11/13	3/11/13	2 hours	Weak.....	6 hours	Hyperaemia pulmonum	No cultures made	—	23/9/13	1	Chick well developed; head of chick away from air chamber.
304	Dead in shell	3/11/13	—	Weak.....	6 hours	Hyperaemia pulmonum	Negative	—	23/9/13	1	Head of chick lying away from air chamber end. Egg broken 3/11/13; dead in shell.
305	3/11/13	3/11/13	A few hours	Weak.....	15 hours	Hyperaemia pulmonum	Negative	—	23/9/13	—	Chick well developed; shell broken to assist chick to hatch.
306	3/11/13	3/11/13	A few hours	Weak.....	15 hours	Hyperaemia and oedema pulmonum	Negative	—	23/9/13	1	Chick well developed; shell broken to assist chick in hatching.
307	3/11/13	3/11/13	A few hours	Weak.....	15 hours	Hyperaemia pulmonum	Negative	—	23/9/13	1	Shell broken to assist chick in hatching; head wrong position.
291	2/11/13	4/11/13	24 hours	Stunted in growth; neck abnormal conformation	4 hours	Negative.....	Negative	—	—	2	
308	3/11/13	4/11/13	24 hours	Weak.....	12 hours	Hyperaemia and oedema pulmonum	Negative	—	23/9/13	1	Shell broken to assist chick in hatching.
309	3/11/13	4/11/13	12 hours	Weak.....	12 hours	Hyperaemia pulmonum	No cultures made	—	—	—	Shell broken to assist chick in hatching.
273	4/11/13	4/11/13	14 days	Stunted in growth; dull for some days prior to death; neck twisted abnormal conformation	12 hours	—	Negative	—	29/9/13	3	

Table No. 5—(continued).

No. of Chick.	Date Hatched.	Age of Chick.	Symptoms.	Interim.	Pathological Anatomical Examination.	Result of Culture Experiments for Aspergillus Injection.	Cause of Death.	Date Egg Incubated.	No. of Incubator.	REMARKS.
322	4/11/13	5/11/13	Weak.....	A few hours	Hyperaemia of small intestines; hyperaemia and oedema pulmonum	No cultures made	—	23/9/13	—	Shell broken to assist chick to hatch; chick well developed.
310	5/11/13	Destroyed 7/11/13	Unable to rise.....	3 hours	Haemorrhagic along back and side of body	Negative	Destroyed (injured)	23/9/13	1	Chick well developed; destroyed for post-mortem.
325	Dead in shell	—	—	25 hours	—	—	—	23/9/13	1	Dead in shell; head lying in wrong position.
311	5/11/13	16/11/13	Morning 16/11/13 noticed lying head on ground, unable to rise	25 hours	Negative.....	No cultures made	—	23/9/13	1	
327	Dead in shell	—	—	—	Hyperaemia pulmonum	No cultures made	—	—	—	Head lying in wrong position.
297	4/11/13	18/11/13	Noticed dull, 17th; on 18th respirations accelerated; chirping noise, not feeding, head carried low and close to body, neck flexed	—	Nodules rare on wall of thoracic air-sac	Aspergillus growth	Aspergillosis	26/9/13	2	
285	29/10/13	23/11/13	Noticed sick 22nd; on 23rd respirations accelerated; not feeding, dull looking, head carried low and close to body, neck flexed	1 hour	Exudate on walls of thoracic air-sac. Oedema pulmonum	Aspergillus growth	Aspergillosis	16/9/13	3	
323	10/11/13	26/11/13	Dull, not feeding, head carried low, legs deformed	6 hours	Hyperaemia pulmonum	Aspergillus growth	Aspergillosis	2/10/13	3	
320	10/11/13	24/11/13	Weak, beak deformed...	—	Hyperaemia pulmonum	No cultures made	—	29/9/13	2	

Table No. 5—(continued).

No. of Chick.	Date Hatched.	Date Died.	Age of Chick.	Symptoms.	Interim.	Pathological Anatomical Examination.	Result of Culture Experiments for Aspergillus Infection.	Cause of Death.	Date Egg Incubated.	No. of Incubator.	REMARKS.
300	4/11/13	27/11/13	23 days	Noticed sick 26th; not moving about, dull, off feed, feels empty	3 hours	Hyperaemia pulmonum	Aspergillus growth	Aspergillosis	23/9/13	1	
337	28/11/13	28/11/13	A few hours	Weak.....	20 hours	Post-mortem changes advanced. Hyperaemia pulmonum	No cultures made	—	16/10/13	2	Shell broken to assist chick in hatching.
332	28/11/13	28/11/13	A few hours	Weak.....	A few hours	Hyperaemia pulmonum	No cultures made	—	16/10/13	3	Shell broken to assist chick in hatching; head lying in wrong position.
333	27/11/13	28/11/13	1 day	Weak.....	A few hours	Hyperaemia pulmonum	No cultures made	—	16/10/13	2	Shell broken to assist chick in hatching; head lying in wrong position.
334	Dead in shell		—	—	—	—	No cultures made	—	16/10/13	3	Chick dead in shell.
336	27/10/13	29/11/13	2 days	Legs deformed; neck twisted, abnormal conformation	A few hours	Hyperaemia pulmonum	No cultures made	—	16/10/13	3	
316	7/11/13	1/12/13	24 days	Noticed sick 30th; dull, off feed, neck flexed, head carried low and close to body, respirations accelerated	A few hours	Hyperaemia pulmonum	Negative	—	2/10/13	3	
324	12/11/13	7/12/13	26 days	Noticed sick for some days; dull, not feeding well, lying frequently	—	Exudate thoracic air-sacs. Hyperaemia pulmonum	Aspergillus growth	Aspergillosis	2/10/13	2	
348	4/1/14	8/1/14	4 days	Noticed sick since 7th; off feed, dull, head carried low and close to body	21 hours	Pleuritis. Hyperaemia pulmonum	Aspergillus growth	Aspergillosis	25/11/13	2	

Table No. 5—(continued).

No. of Chick.	Date Hatched.	Date Died.	Age of Chick.	Symptoms.	Interim.	Pathological Anatomical Examination.	Result of Culture Experiments for Aspergillus Infection.	Cause of Death.	Date Egg Incubated.	No. of Incubator.	REMARKS.
350	4/1/14	8/1/14	4 days	Head low, neck flexed, off feed, dull	5 hours	Hyperaemia pulmonum	Aspergillus growth	Aspergillosis	25/11/13	3	
354	9/1/14	12/1/14	3 days	Yolk-sac protruding....	A few hours	Hyperaemia pulmonum	Negative	—	1/12/13	1	
355	11/1/14	12/1/14	1 day	Weak.....	A few hours	Hyperaemia pulmonum	Negative	—	1/12/13	1	
357	11/1/14	14/1/14	3 days	Respirations accelerated, off feed, dull, neck flexed, head carried low	—	Hyperaemia pulmonum	Aspergillus growth	Aspergillosis	1/12/13	1	
347	3/1/14	16/1/14	13 days	Respirations accelerated, off feed, dull, neck flexed, head carried low, temperature sub-normal	A few hours	Nodules frequent in lungs and on walls of thoracic air-sacs	Aspergillus growth	Aspergillosis	25/11/13	2	
349	3/1/14	16/1/14	13 days	Stunted, neck flexed, head carried low, off feed	—	Hyperaemia pulmonum, Cytomycosis	Aspergillus growth	Aspergillosis	25/11/13	1	
353	11/1/14	21/1/14	10 days	Noticed sick since 18th; respirations accelerated, off feed, dull, head carried low and close to body, neck flexed, temperature sub-normal from 19th	4 hours	Hyperaemia pulmonum, Tubercles in lungs and on wall of air-sacs (thoracic)	Aspergillus growth	Aspergillosis	1/12/13	1	

Résumé.

Total number of deaths, 43.

11 chicks, ages from 3 to 26 days, showed symptoms of aspergillosis, cultural experiments confirming the existence of aspergillus infection.

14 chicks died within from a few hours to 36 hours of hatching. In each instance the shell had to be broken to assist in the hatching. (No cultures were made).

5 chicks were found dead in the shell. (No cultures were made).

8 chicks died within from 24 hours to 25 days of hatching.

Cultural experiments gave negative results for aspergillosis.

1 chick was destroyed for post-mortem. Cultural experiments negative.

4 chicks, deformed at time of hatching, negative for aspergillosis.

Total...43

(5) *Experiments to ascertain Results of Infecting the Air Chamber of Incubating Ostrich Eggs with Spores of Aspergillus fumigatus.*

(For details see Table No. 6).

Table No. 6.
INFECTION OF OSTRICH EGGS EXPERIMENT.

Egg No.	Date Inoculated.	Method.	Inoculated.	Incubator No.	Result.	Cultural Experiments for the Recovery of <i>Aspergillus fumigatus</i> from Inoculated Eggs.
1	7/11/13	A small opening was made in the shell, <i>Aspergillus fumigatus</i> , from a culture on potato introduced into the air chambers and gummed paper placed over the opening and melted paraffin wax poured over the paper.	5/11/13	1	Examined and opened on 14/11/13; result, egg contents decomposed	Aspergillus recovered from air chamber.
2	7/11/13		5/11/13	1	Examined and opened on 17/11/13; result, egg contents decomposed	" "
3	17/11/13		5/11/13	1	Examined and opened on 27/11/13; result, contents decomposed	" "
4	17/11/13		5/11/13	1	" " " " " "	" "
5	27/11/13		5/11/13	1	Examined and opened on 2/12/13; result, contents decomposed	" "
6	27/11/13		5/11/13	1	Examined and opened on 2/12/13; chick dead.....	" "
7	8/12/13		5/11/13	1	Examined and opened on 15/12/13, and chick found dead in shell	" "
8	8/12/13		5/11/13	1	Examined and opened on 10/12/13; chick alive.....	" "
9	13/12/13		5/11/13	1	Chick found living with head in air chamber when shell opened to inoculate with <i>Aspergillus</i> spores; consequently spores, when blown into shell, must have been inhaled by the chick. Egg opened on 15/12/13 and chick found dead	Aspergillus recovered from air chamber and lung tissue.
10	13/12/13		5/11/13	1	Examined and opened on 15/12/13, and chick found dead	Aspergillus recovered from air chamber.

Résumé.

The air chamber of ten normal ostrich eggs was inoculated on the 2nd, 12th, 22nd, 33rd, and 38th day after inoculation with *Aspergillus fumigatus*. The contents of five were examined on the 7th, 10th, 22nd, day, respectively, after inoculation and found to be decomposed. Four contained a dead chick and the remaining egg was opened and chick found alive.

Aspergillus fumigatus was recovered in culture from the air chamber of each egg and from lung tissue of the chick, which was found alive with its head in the air chamber when the egg was inoculated.

(6) *Experiments to ascertain whether the Incubators were Infected.*

(For details see Table No. 7.)

Table No. 7.

CULTURES MADE FROM INCUBATORS.

Date.	No. of Incubator.	Method.	Period Medium kept in Incubator.	RESULT.
19/1/14	1	Potato medium placed in sterile petri dish in incubator for hours, and then potato put back in test tubes and placed in incubator at 37°	24 hours	Growth of <i>Aspergillus fumigatus</i> and <i>Aspergillus</i> (species ?).
19/1/14	2		"	" " "
19/1/14	3		"	Growth of <i>Aspergillus fumigatus</i> .
27/1/14	1		"	Growth of <i>Aspergillus fumigatus</i> and a fungus with a sterile mycelium.
27/1/14	2		"	Growth of <i>Aspergillus fumigatus</i> and other species.
27/1/14	3		"	Growth of <i>Aspergillus fumigatus</i> and other species.

Résumé.

Potato medium placed for twenty-four hours in the incubators which were in use during the seasons 1913-14, and then incubated at incubator temperature showed infection with *Aspergillus fumigatus* and other species and other fungi.

(7) *Experiment to ascertain Result of Infecting Shells of Fowls' Eggs with Spores of Aspergillus Fumigatus and whether Clean Eggs placed in the same Incubator would become Affected.*

(For details see Table No. 8.)

Table No. 8.
EXPERIMENTS WITH FOWLS' EGGS.

Egg No.	Date of Experiment.	Experiment.	Date of Incubation.	RESULT.
40	27/6/14	Smeared on air chamber end of shell, potato culture of <i>Asp. fumigatus</i>	16/6/14	Chick dead; <i>Asp. fumigatus</i> infection in air chamber.
43	"	" " "	"	Hatched 7/7/14.
51	"	" " "	"	Unfertile.
62	"	" " "	"	Chick dead; <i>Asp. fumigatus</i> infection in air chamber.
60	"	" " "	"	Unfertile; <i>Asp. fumigatus</i> infection in air chamber.
59	"	" " "	"	" " "
56	"	Incubated in same incubator with above	—	Hatched 7/7/14.
52	"	" " "	—	Unfertile.
61	"	" " "	—	Unfertile; <i>Asp. fumigatus</i> infection in air chamber.
64	"	" " "	—	Unfertile.
53	"	" " "	—	"
65	"	" " "	—	Dead chick.
47	1/7/14	" " "	—	Dead chick; <i>Asp. fumigatus</i> infection in air chamber.
54	"	" " "	—	" " "
56	"	" " "	—	Hatched 7/7/14.
57	"	" " "	—	"
58	"	" " "	—	Dead chick; <i>Asp. fumigatus</i> infection in air chamber.
68	"	" " "	—	Fungus infection; not <i>Aspergillus</i> on shell membrane.
55	"	" " "	—	Unfertile, contents decomposed; <i>Asp. fumigatus</i> infection in air chamber.
63	"	" " "	—	" " "
67	"	" " "	—	Unfertile.
69	"	" " "	—	Hatched 7/7/14.
66	"	" " "	—	"

Résumé.

The unbroken shells of the six fowl eggs were infected with potato culture of *Aspergillus fumigatus* and the eggs incubated, with the result that the air chamber of four of these became infected with *Aspergillus fumigatus*. (Two of the eggs contained a dead chick.)

The remaining two eggs were not infected. (One chick hatched, the other egg was unfertile.)

Of the seventeen eggs incubated with the above, the air chamber of—

6 was infected with *Aspergillus fumigatus* (3 contained a dead chick, 3 were unfertile);

5 produced chicks and were not infected;

4 were unfertile and were not infected;

1 showed infection of a fungus growth on shell membrane (not *Aspergillus*); and

1 contained dead chick (egg not infected with *Aspergillus*).

Total...17

(8) *Experiments to Note whether Spores of Aspergillus Fumigatus Dosed to an Ostrich would Vegetate after Passing through the Intestinal Canal.*

(For details see Table No. 9.)

Table No. 9.

No. of Experiment.	Date of Experiment.	No. of Ostrich in Experiment.	Age of Ostrich.	Material and Quantity Dosed.	Date Medium Inoculated.	RESULT.
1	6/1/14	331	56 days	200 mg. of a potato culture	7/1/14	No growth of <i>Asp. fumigatus</i> .
					8/1/14	" " "
					9/1/14	Growth of <i>Asp. fumigatus</i> .
					10/1/14	" " "
					11/1/14	No growth of <i>Asp. fumigatus</i> .
					12/1/14	" " "
2	15/1/14	40	Adult	200 mg. of a potato culture	14/1/14	No growth of <i>Asp. fumigatus</i> .
					15/1/14	" " "
					16/1/14	" " "
					17/1/14	Growth of <i>Asp. fumigatus</i> .
					18/1/14	" " "
					19/1/14	No growth of <i>Asp. fumigatus</i> .
3	16/1/14	331	66 days	200 mg. of a potato culture	15/1/14	No growth of <i>Asp. fumigatus</i> .
					16/1/14	" " "
					17/1/14	Growth of <i>Asp. fumigatus</i> .
					19/1/14	No growth of <i>Asp. fumigatus</i> .

Résumé.

The faeces of an ostrich dosed with spores of *Aspergillus fumigatus* produced in the first experiments a growth of *Aspergillus fumigatus* on the 3rd and 4th days, in the second experiment on the 2nd and 3rd day, and in the third experiment on the 3rd day, respectively, after dosing.

(9) *Experiment to Note whether the Faeces of an Ostrich which was being fed on Lucerne would produce a Growth of Aspergillus Fumigatus.*

(For details see Table No. 10.)

Table No. 10.

No. of Experiment.	Date of Experiment.	No. of Chick in Experiment.	Age of Chick.	Method.	RESULT.
1	24/1/14	—	5-6 months	Faeces were collected from the cloaca and inoculated on potato medium	Growth of <i>Asp. fumigatus</i> and other fungi.
2	28/1/14	—	"		" " "
3	29/1/14	—	"		" " "

Résumé.

Faeces collected from an ostrich being fed on lucerne produced a growth of *Aspergillus fumigatus* in three experiments.

(10) *Experiments to Note whether Lucerne (and Soil) collected from a Field the Lucerne of which was being used for Feeding Chicks.*

(For details see Table No. 11.)

Table No. 11.

Field from which lucerne collected had been manured for some years with stable litter, straw, etc.

Experiment No.	Date.	Material.	Inoculated.	RESULT.
1	19/1/14	Lucerne.....	—	<i>Aspergillus</i> growth.
2	19/1/14	Lucerne leaves from lucerne patches for feeding chicks	—	" "
3	19/1/14	Soil from lucerne field.....	—	" "

Résumé.

Lucerne and soil collected from a field which had been fertilized for some considerable time with stable manure produced a growth of *Aspergillus fumigatus* and other species of *Aspergillus* and other fungi.

STERILIZATION EXPERIMENTS.

Date of Experiment.	EXPERIMENT.	RESULT.
1/8/14	5 per cent. carb. acid solution kept for half-hour in contact, in culture tube, with potato culture of <i>Aspergillus fumigatus</i> and sterile potato media, then inoculated from above culture	Culture not sterilized.
1/8/14	1:1000 corrosive sublimate solution kept for half-hour in contact, in culture tube, with potato culture of <i>Aspergillus fumigatus</i> and sterile potato media, then inoculated from above culture	Culture not sterilized.
1/8/14	Potato media inoculated with <i>Aspergillus fumigatus</i> and culture tubes kept for half-hour in boiling water sterile potato media, then inoculated from above culture	Culture sterilized.
1/8/14	Potato media inoculated with <i>Aspergillus fumigatus</i> and cultures autoclaved at 120° C. for half-hour and sterile potato media, then inoculated from above culture	Culture sterilized.
1/8/14	Box infected with <i>Aspergillus fumigatus</i> and sealed for half-hour in 1:1000 corrosive sublimate solution and sterile potato media placed in box	Box not sterilized.

Numerous observations have shown that certain fungi belonging to the group Eumycetes are pathogenic for birds and cause an extensive pathological process, as in the case of pulmonary mycoses (pneumo mycosis) or in a generalized infection in which the spores are probably carried by the blood or lymph stream, lodging in various parts of the body and producing serious lesions, and although in many cases no careful species examination was made, in all probability *Aspergillus fumigatus* was responsible.

Occurrence.—It is particularly among avians that aspergillosis is observed. Apparently the first case was observed by Meyer and Esnert in a jay in 1815. Since then its presence has been recorded many times in various domesticated and wild birds, such as the pigeon, turkey, common domesticated fowl, goose, duck, swan, flamingo, seagull, stork, ostrich, plover, owl, etc. Its existence was first noted by the writer in South Africa in an adult ostrich in 1912, and later in chicks of various ages. Jowett¹ recorded cases in the adult ostrich in the Cape Province, and concluded the disease was probably contracted through eating mouldy food. Its occurrence has been observed by Balfour in a turkey² in the Sudan, and Archibald recorded a case in the Sudan ostrich.³

Symptoms: Duration and Course.—In young chicks of a few days old the first indication that there is something amiss is a disinclination to feed. When watched closely it will be noted that the chick either picks at the food without taking any in the beak or lifts some from the ground and allows it to fall. Chick appears dull and weak, eyes half-closed, moves about slowly or stands and frequently utters a plaintive note. The neck is usually flexed and the head lowered and kept close to the body. Fig. 1. The abdomen soon loses the tense and full feeling found in healthy chicks; the respiration may or may not be visibly accelerated; in some cases, at the later stages, the beak is partly opened from time to time, a long inspiration being taken; in other instances the beak was found to be kept continually open.

In visibly affected chicks the temperature was found to be irregular, a characteristic being the marked variations between the morning and evening temperatures, 3° to 5° F. (in healthy chicks the rule is to find a difference of about 2° F., 102°–104° F.).

In some instances small whitish nodules, averaging in size that of a pin's head, were detected on examination of the buccal mucous membrane, epiglottis, and other positions in the mouth. Death is usually ushered in with a pronounced fall of temperature.

Duration and Course.—Death occurs in a few days from the appearance of the symptoms.

Mortality.—It is the exception to find an affected chick recover.

¹ Jowett, W. "Pulmonary mycosis in the Ostrich." *Journal of Comparative Pathology and Therapeutics*, Vol. XXVI, Part 3, September, 1913.

² Balfour, A.: "Aspergillary Pneumo-mycosis in the Lung of a Turkey." *Journal Report, Wellcome Tropical Research Laboratories*, 1911.

³ Archibald, Capt. R. G.: "Aspergillosis in the Sudan Ostrich." *Journal of Comparative Pathology and Therapeutics*, Vol. XXVI, Part 2, June, 1913.

In *adult birds* marasmus is sometimes the only sign of the malady. When the lungs are affected respirations are accelerated, the beak being frequently kept partly open. When the liver is chiefly involved there is usually a pronounced loss of condition. In adult birds, as a rule, the disease runs a sub-acute or chronic course.

Lesions and Seat of Lesions.—Whereas in adult birds the disease was frequently found to be more or less generalized (the respiratory tract and liver being the most constant seat of lesions), in young chicks, in cases of natural infection, lesions were chiefly confined to the respiratory tract (lungs and air-sacs). In some instances nodules were observed on the buccal membranes, epiglottis, and other positions in the mouth.

It is usual to find on close examination of the lungs of the latter at least some nodules more or less rounded in shape and of a yellowish white colour, varying in size—average, 1·2 mm. In rare cases a caseous focus or a pneumonic patch was noted.

When existing in the walls of the air-sacs the lesions consist of small tubercles and sometimes a fibrinous exudate.

In adult birds degenerative changes of the tubercles and destructive metamorphosis in the surrounding tissues are marked.

In one instance the writer observed the mycelium and conidial development of *Aspergillus fumigatus* in the air cavities of the bone of the sternum and in another that of *Aspergillus* species nearest to *Asper. niger* in the thoracic air-sac.

The lesions found in experimentally produced cases were as follows:—

Intravenous injection of spores produced a more or less generalized infection, the lungs and liver being particularly involved and showed disseminated tubercles.

Inhalation of Spores.—The lesions were marked in the lungs and air-sacs (thoracic). In the former they assumed the form of dirty whitish nodules, disseminated or agglomerated, and in the latter numerous tubercles were formed on the inner surface of the walls.

The ingestion of cultures resulted in no marked intestinal changes, but in some instances tubercles were found in the lungs.

Histopathology (natural infection).—The reaction on the part of the tissue manifests itself in the formation of tubercles composed of epithelioid cells and leucocytes and some eosinophile cells, and amongst which it is usual to find some mycelial filaments. Owing to the short duration of the disease in young chicks, it is unusual to find caseous degeneration and fibrous transformation of the tubercles; in rare instances a caseous focus and a pneumonic infiltration of the surrounding lung tissue was seen. In *adult birds* the tubercles may present all the stages of evolution of typical tubercle, viz., miliary granulations, caseous degeneration, and fibrous transformation. In the liver it is usual to find extensive caseous areas resulting from degeneration of the tubercles which are usually found agglomerated in this organ, as well as necrosis of the surrounding parenchyma.

Diagnosis.—Outbreaks of chick fever usually assume an epidemic form and occur amongst chicks from a few days up to 3–4 weeks after hatching; the respirations are frequently accelerated and the beak may

sometimes be kept partly open. On examination of the buccal membrane, small whitish nodules, averaging in size that of a pin's head, are sometimes found. The symptoms previously described and history assist in the diagnosis.

A careful post-mortem examination of the lungs and wall of the thoracic air-sacs usually reveals some nodules, which, when inoculated on potato medium and incubated at 35° C., produce small whitish growths, visible on examination with the naked eye, at about the 24th–36th hour after incubation. After about another 24 hours the colour appears bluish and later turns dark bluish green.

In some instances no nodules were detected, but cultures on potato made from portions of hyperaemia lung tissue produced a growth.

Conclusions.

1. *Aspergillus fumigatus* appears in the ostrich, more particularly in the ostrich chick from a few days to about 3–4 weeks after hatching, and is responsible for the disease in ostrich chicks commonly known as “Yellow Liver or Chick Fever.”

2. *Aspergillus fumigatus* is the commoner and more pathogenic species.

3. Outbreaks usually appear in an epidemic form and are more prevalent amongst chicks artificially hatched and reared.

4. *Aspergillus fumigatus* infection occurs in the air chamber of the egg, and is common in straw and other vegetable matter and in soil which has been fertilized with decomposed vegetable matter, such as stable manure, etc.

5. Infected eggs are the chief source of infection of incubators, the liberation of *Aspergillus fumigatus* from the air-chamber taking place either at the time of hatching or when infected eggs are opened in the incubators.

6. Aspergillosis is contracted naturally from—

(a) Infected eggs just previous to or at time of hatching.

(b) Infected bedding used in chicks' sleeping boxes.

(c) Infected incubators.

7. Aspergillosis may be transmitted artificially by inhalation and ingestion and intravenous inoculation of cultures.

8. *Aspergillus* infection occurs chiefly through the respiratory tract, the lungs and air-sacs being the seat of infection.

9. Infection may occur through the digestive tract.

10. *Aspergillus fumigatus* is transmitted from infected to clean eggs through the unbroken shell.

11. The contents of unbroken eggs may escape through the intact shell. In such cases the bacteria which exist in the contents are probably a source of infection of eggs, more particularly those in contact.

12. Spores of *Aspergillus fumigatus* vegetate after passing through the intestinal canal.

Prevention consists in the use of:

(1) Non-infected incubators.

(2) Non-infected bedding in the chicks' sleeping boxes.

(3) Non-infected eggs for incubation purposes.

Boiling water has given satisfactory results in the sterilization of cultures of *Aspergillus fumigatus*.

Temperature Charts.

1. Temperature chart of a normal chick, age 4 months.
2. Temperature chart, a chick which contracted aspergillosis from infected straw.
3. Temperature chart of a chick which contracted aspergillosis from dosing with *Aspergillus fumigatus*.
4. Temperature chart of aspergillosis (natural infection).
5. Temperature chart of aspergillosis experimentally produced by inhalation of *Aspergillus fumigatus*.

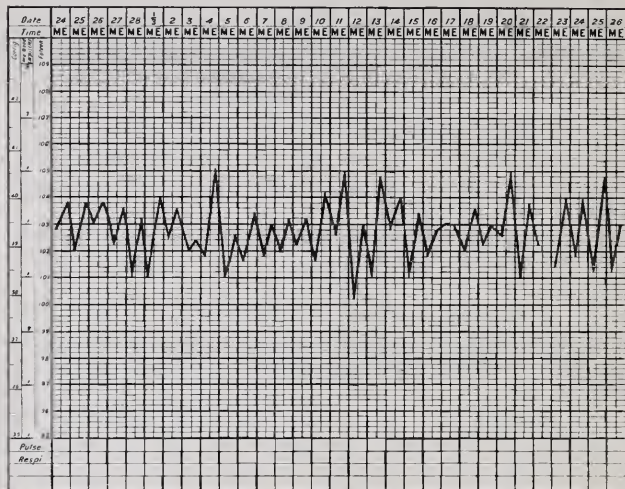
-NORMAL CHICK, AGE FOUR MONTHS.

February, 1914.

Animal.

OSTRICH CHICK.

No. 147.



ASPERGILLOSIS CONTRACTED FROM INFECTED STRAW IN CHICK BOXES.

November, 1913.

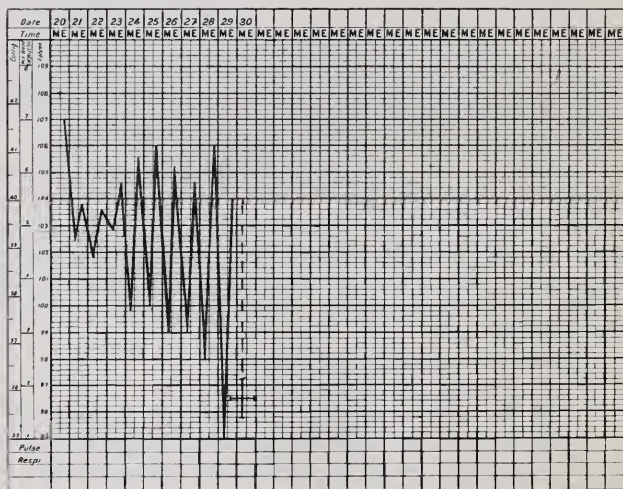
Animal.

OSTRICH CHICK

No. 322.

Remarks.

Hen No. 16—
hatched 10/11/13.



CHICK DOSED WITH ASPERGILLUS FUMIGATUS SPORES.

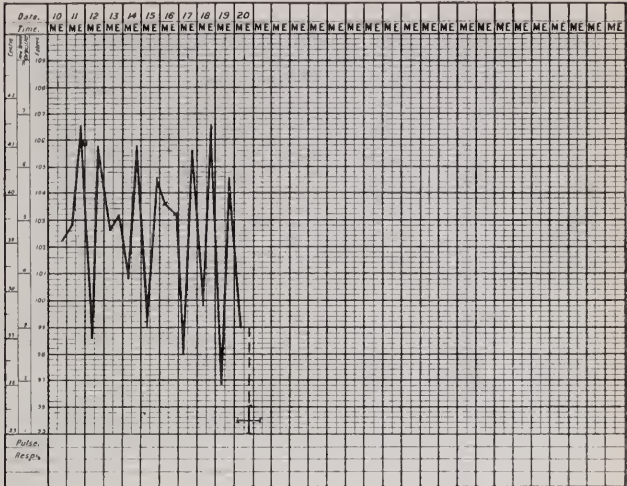
November, 1913.

Animal.

OSTRICH CHICK.

No. 301.

Remarks.

Hatched 4/11/13;
died 20/11/13.

ASPERGILLOSIS NATURAL INFECTION.

January, 1914.

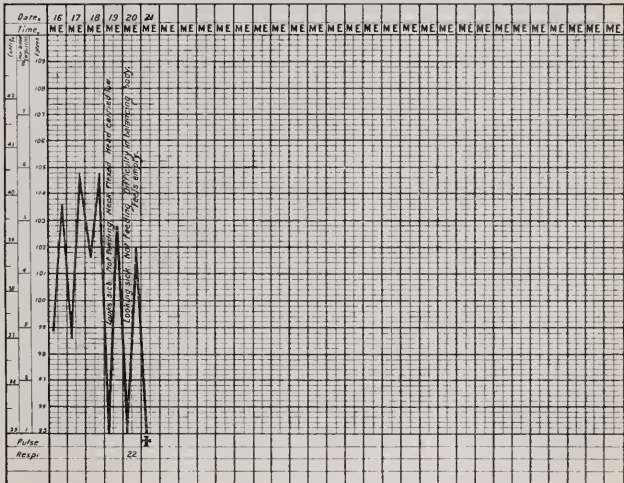
Animal.

OSTRICH CHICK.

No. 353.

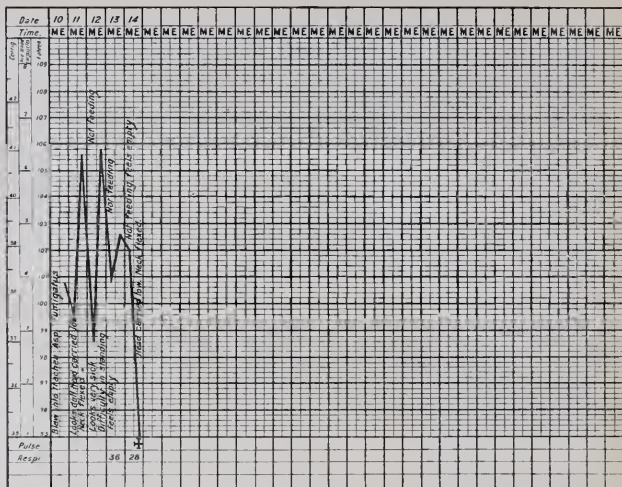
Remarks.

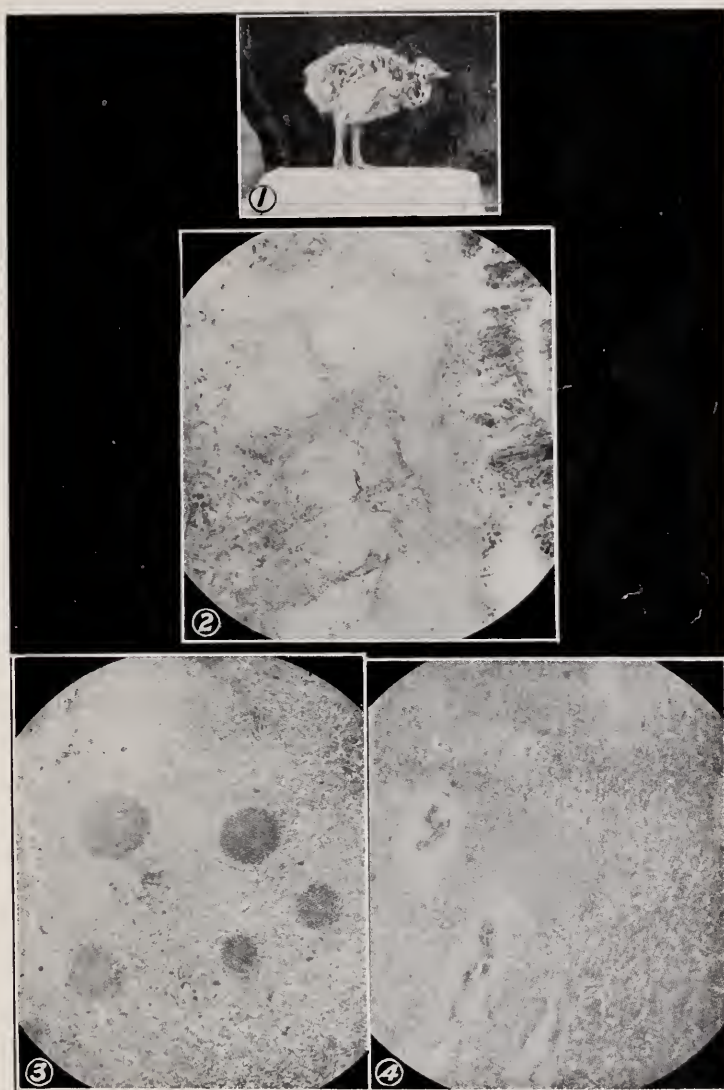
Hatched 11/1/14.



OSTRICH CHICK.
No. 299.

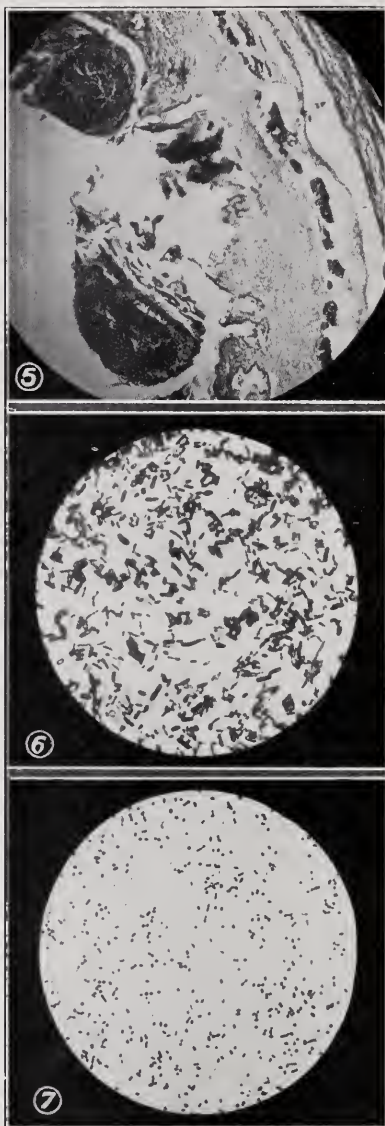
Hatched 4/11/13.





Aspergilliosis.

J. Walker



Aspergillosis

J. Walker.

**A Short Note on the occurrence of
Cytodites nudus (Vizioli) in the
Domestic Fowl in South Africa.**

BY

JAMES WALKER,

Veterinary Research Laboratory, Grahamstown.

A Short Note on the occurrence of *Cytodites nudus* (Vizioli) in the Domestic Fowl in South Africa.

By JAMES WALKER, Veterinary Research Laboratory, Grahamstown.

INTRODUCTION.

PARASITES belonging to the genus *Cytodites* were first seen in birds in 1859 by Gerlach, who referred them to the genus *Sarcoptes*. Megnin established the genus in 1877 and separated it from that of *Sarcoptes*. Only one species appears to be known, "*The Cytodites nudus*." On referring to Professor Neumann's "*Treatise on the Parasites and Parasitic Diseases of the Domesticated Animals*," page 244, I find that Gerlach has accused them of causing enteritis amongst poultry, and Zundel believed they caused enteritis and peritonitis. Zschokke saw them in the lungs, trachea and anterior air sacs surrounded by yellow gelatinous masses, deposits of false membranes, and a quantity of mucus, but he was not certain whether there was not a simple coincidence between their presence and the deposits of false membranes, etc.

Holzendorff made post-mortems on several fowls from the same poultry yard which had been ill for a long time, and found in the lungs, liver, kidneys, etc., a number of yellow miliary nodules; and in the thorax many cytodites which were also recognized in the tubercles. Megnin has seen them on one occasion cause death by obstruction and consequent asphyxia. It appears, however, that the cytodites may exist in large numbers in the air sacs and produce no ill-effects, and it is only when they are extremely numerous and in the bronchi that they may cause fits of coughing due to irritation of the mucous membrane.

So far as the writer is aware the occurrence of this parasite has not previously been noted in the domestic fowl in South Africa. In January, 1913, the writer was requested to investigate the cause of the mortality occurring in some domestic fowls belonging to a resident of Grahamstown, Cape Province. The following symptoms were noted by the owner:—Loss of appetite, comb turning purple, fowl seems fretful and weak in its legs, body very much tucked up; the birds are noticed sick for a week, then there is a sudden collapse; total deaths ten to twelve. Unfortunately I had no opportunity of examining a sick bird. Two dead fowls were sent to the Laboratory. The following is a record of the post-mortem examinations:—

P.M. No. 551. Date of Death: 7th January, 1913. Place: Laboratory, Grahamstown. Distinctive No., 7. Species: Domestic fowl.

Age: Full grown. Interim: Several hours. Condition: Good. Integument: Normal. Natural openings: Normal.

Visible mucous membranes: Normal. Appearance of flesh: Normal.

Subcutaneous tissues: Normal. Thorax: Cytodites frequent.

Peritoneal cavities: Cytodites frequent.

Mesentery: Cytodites fairly frequent. *Thoracic and abdominal air sacs*: Cytodites fairly frequent. *Larynx*: Normal.

Trachea: Cytodites fairly frequent. *Lungs*: Mottled in appearance, some purple patches, size .2-.3 cm. in diameter. On section hyperaemia a serous fluid exuded from the cut surface, scattered throughout the affected portion of the lung are small miliary tubercles of a gelatinous consistency, in the midst of these the parasites are visible.

The larger bronchi and some of the bronchioles contain an exudate in which the parasites are embedded.

Heart: Normal. *Endocards*: Normal. *Epicard*: Normal.

Liver: Normal. *Spleen*: Normal. *Proventriculus mucous membrane*: Normal. *Gizzard*: Normal. *Small intestines*: Normal.

Large intestines: Normal. *Kidneys*: Normal.

Microscopical examination of the blood: Negative.

Pathological Anatomical Diagnosis.—Hyperaemia and oedema of lungs and broncho-pneumonia.

Fowl No. 6, sent in by the same owner on the 7th January, 1913, showed on post-mortem examination a similar pathological condition of the lungs, with cytodites in the trachea, bronchi, thoracic and abdominal air sacs, thoracic and peritoneal cavities.

The parasites were identified by G. A. H. Bedford, of this division. They are readily seen with the naked eye, and correspond with the description given in Professor Neumann's "Treatise of Parasites and Parasitic Diseases of the Domestic Animals," page 243, which reads as follows:—Colour whitish, body smooth and nearly glabrous, prolonged in front by a conical rostrum which forms a tubular sucker. Legs composed of five articles disposed as in the sarcoptes, and terminated in an ambulacrous sucker with a simple pedicle. The male measures about .45 mm. long and .15 mm. broad, these being apparently younger forms.

Pathological Appearances.

To the naked eye.—The lung tissue has a mottled appearance. The surfaces show some purple patches about .2-.3 cm. in diameter.

On section the lung tissue is hyperaemic. A serous fluid exudes from the cut surface. Scattered throughout those parts of the lung in which the parasites are frequent small miliary tubercles, size of a pin's head, and of a gelatinous consistency, occur. Parasites are visible in the tubercles and also in the invaded air conduits.

Sections were fixed in 4 per cent. formalin sol, embedded in paraffin, and stained with giemsa and with haematoxylin and Von Geison stains.

300 Magnification.—Instead of the open network seen in the normal lung of a bird (fig. 1), the lung tissue surrounding the invaded air passages is infiltrated with cells (leucocytes and red blood corpuscles), the blood vessels are congested (fig. 2), some of the parasites have wandered from the air conduits into the surrounding parenchyma, breaking down the regular network conformation of the latter, in some of the invaded air passages the parasites have lodged themselves in and broken down the inner lining (fig. 3). Cytodites are also to be seen in

the interlobular fibrous tissue in places. The larger bronchi contain an exudate.

Conclusions.—*Cytodites nudus* occurs in South Africa in the domestic fowl. In the two instances in which post-mortem examinations were made the parasites were evidently responsible for the lesions found in the lungs which, in the writer's opinion, caused the death of the birds.

Micro-photographs:—

1. Normal portion of lung of fowl No. 7.
2. Portion of affected lung of fowl No. 7. Infiltrated with cells and showing congested blood vessels.
3. An air passage, fowl No. 7, showing cytodites.



Cytodites nuchus.

J. Walker.

Investigations into Jagziekte or Chronic Catarrhal-Pneumonia of Sheep.

By

D. T. MITCHELL,

Veterinary Research Laboratories, Onderstepoort.

Investigations into Jagziekte or Chronic Catarrhal-Pneumonia of Sheep.

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PART I.

CHRONIC CATARRHAL PNEUMONIA OF SHEEP—JAGZIEKTE.

UNDER this heading Hutcheon, in a pamphlet dealing with diseases of stock in South Africa, describes a form of inflammation of the lungs affecting sheep in the Cape Colony, particularly in the colder districts at a considerable altitude above the sea-level.

The appearances presented resembled catarrhal pneumonia, and the lesion showed a great tendency to terminate in interstitial pneumonia. Abscess formation and breaking down of the lung tissue was rarely seen in uncomplicated cases. In advanced cases the pulmonary tissue was obliterated and the lesion became drier, hard, and elastic. No tendency to recovery was observed even when the affected animals were housed.

The symptoms shown were coughing, which became more aggravated as the disease advanced, and very much accelerated respirations, which in the later stages became so pronounced that the animal appeared to have been rapidly driven, hence the Dutch name jagziekte (jagt, to drive; ziekte, a sickness).

A histological examination appears to have been carried out, and attention is drawn to the closing up of the smaller bronchi and air-cells by pressure from the hypertrophy of the lung tissue, and to the fact that little catarrhal products were demonstrated in the alveoli and bronchi.

The disease was considered to be infectious "under certain climatic conditions," but attempts at artificial transmission by cohabitation and inoculation with blood and inflammatory products were unsuccessful.

Robertson in 1904 further described the disease to be "due to some specific other than exposure to cold and wet."

The symptoms described correspond to those described by Hutcheon. The effect of careful treatment and nursing is pointed out as being effectual in prolonging the animal's life, and it is stated that the course of the disease is more rapid in winter than in summer. Slaughter of all affected animals is recommended as a prophylactic treatment.

The post-mortem appearances of the affected lungs are described as follows:—

"The lungs and their attendant lymphatic glands are alone affected. The latter are enlarged, pale, and oedematous. The lungs show more or less extensive areas of consolidation—sometimes there seems to be one centre or area of infection, but other cases seem to indicate that the infection has started from many centres. The affected part is quite solid, of a darker colour than normal lung, firm, friable,

and slippery to the touch. In some cases the affected part cuts like bacon, and the cut surface has a soapy, greasy feeling to the fingers. In advanced cases fibrous tissue may form to such an extent that the affected part of the lung cuts like cartilage.

“The bronchi persist, but the lumen is often occluded.

“The consolidated portions do not appear to be either congested or swollen. On squeezing the cut surface a quantity of froth and débris oozes from the bronchi. The plugged air cells do not empty when scraped. Sometimes on section the plugged bronchi stand out like tubercles and the lungs may be as dry as cartilage.”

As pointed out by Hutcheon, no tendency to abscess formation or breaking down of the lung tissue was observed.

Lesions of pleurisy were noticed occurring when the pulmonary lesions were well advanced; only the pleura over the affected part was involved.

It was noted that the lesions in the lung were sharply defined and separated from the surrounding unaltered or only slightly altered lung tissue by a well-marked line of demarcation.

Robertson described crescent-shaped bodies with rounded ends which he found present in smears made from lung lesions. These bodies he considered of a protozoon nature, and he thought that they resembled crescents of sub-tertian malaria, but, although they were only found in smears made from lesions in the lungs of sheep affected with chronic catarrhal pneumonia and were absent from smears made from the healthy areas of these lungs or from the lungs of healthy sheep, he did not feel justified in considering these to be the causal parasite of the disease.

He failed to convey the disease by means of artificial transmission, including “cohabitation, feeding, and all varieties of inoculation methods with blood and affected tissue.”

In a further article by Hutcheon with reference to this disease in 1905, it is stated that the disease may also affect goats. The remainder of the article dealt with points already described in previous articles, and nothing further of interest was recorded. Subsequent to this date nothing seems to have been written regarding this disease.

Various experiments were undertaken at the Grahamstown Bacteriological Institute and at the Bacteriological Laboratory, Onderstepoort, but the results were disappointing. Owing to reports of severe outbreaks appearing in some of the northern districts of the Cape Province in 1911-12, investigation work was begun on a large scale at the Veterinary Research Laboratory, Pretoria, with a view to elucidating the cause and the collection of information regarding the incidence, etc., of the disease.

The information regarding the first appearance of the disease is difficult to obtain, as it is unusual for accurate records of losses among small stock to be kept, and undoubtedly many of the earlier cases were mistaken for other diseases which caused marked loss of condition, such as worm infection, or, where the flocks were large and careful supervision not carried out, the cases escaped notice. Inquiries from sheep farmers at present in the affected area go to show that the disease has been known and recognized since 1893, and a few state that they have known the disease for upwards of thirty years.

The exact distribution of the disease is as yet not fully known, and there is reason to believe that it occurs in many districts from which no report of its occurrence has yet been received. This con-

clusion must be arrived at when the geographical distribution of the known infected areas is studied.

Up to the present cases have been reported from the following districts:—

Cape Province.—Steynsburg, Philipstown, Port Elizabeth, and Willoughby Districts.

Orange Free State.—Smithfield, Bethulie, Winburg Districts.

Transvaal.—Piet Retief, Krugersdorp, Standerton Districts.

All breeds of sheep appear to be equally susceptible. Cases of a disease with similar symptoms have been reported occurring in Angora goats, but as no cases in goats have yet been investigated it is not possible to say whether the condition is similar to that found in sheep or not.

The disease occurs in a sporadic form. Experimental work in connection with artificial transmission of the disease has proved that the lesions or discharges of animals found on post-mortem to be suffering from the disease are not infective, either when inoculated into healthy animals or when drenched. Conclusive evidence is, however, on hand to prove that the disease can be conveyed to healthy animals by cohabitation or immediate contact with infected sheep, and also by kraals in which infected animals have been kept for some time and removed previous to the introduction of healthy animals. In one respect, that of direct contagion, the conditions of infection resemble those of pleuro-pneumonia contagiosa of cattle, and point to the presence of a fixed virus, although up to the present no organisms have been found in the lesions or discharges which could be looked upon as the causal agent. It is reasonable to suppose that the infection may be transmitted by inhalation, but experimental proof on this point is still wanting.

The disease mostly occurs in sheep about three years old. Cases have been reported in sheep eighteen months old, but no cases have been noted to occur among lambs. Young vigorous sheep in good condition do not usually become infected under natural veld conditions, but no difference in susceptibility due to age or condition could be observed among the experimental animals.

In regard to the effect of sex, opinions of the farmers seem to be divided—but no differences in susceptibility in the sexes was apparent during the experiments.

Persian, Merino, and Africander sheep, the breeds most commonly met with in South Africa, seem to show no difference in regard to their susceptibility to the disease.

The initial symptoms are more readily seen in Merinos owing to their longer fleece aggravating the respiratory symptoms. Imported sheep contract the disease, but do not appear to be more susceptible than colonial-bred animals.

The greatest mortality from the disease occurs in particularly wet years, and more cases develop in the flock during the rainy season than during the dry season. This is most probably due to the rain and subsequent evaporation causing loss of body-heat and predisposing to "chill," and thereby lowering the resistance of the body.

In a disease such as this, which is transmitted by contact, one would expect to find that cases would be more frequent among flocks which were kraaled at night, as this practice would greatly increase the opportunities for infection to be conveyed from diseased to healthy

animals. This is found to be the case in most instances, although some farmers assert that cases are not diminished in flocks which are not kraaled. This diversity of opinion can only be explained by the fact that other predisposing causes were not taken into account, and in a disease which shows marked seasonal or yearly variation it is difficult for farmers to give a definite opinion on this point. In most cases where an investigation into the conditions was made it was found that either the sheep were not kraaled, or only kraaled for certain periods of the year—for example, during lambing season. This fact may account for the comparatively low mortality which occurs in infected flocks under natural conditions.

The water supply on infected farms was obtained from various sources. In some cases the supply was from a dam, in others from a river or borehole. The mortality showed that the incidence of the disease had no connection with the source of supply or the method of watering the animals. This was confirmed later during the experimental work carried out in connection with the disease, when it was found that drenching of an emulsion made from the affected part of the lungs of animals suffering from the disease, or with catarrhal discharge from the trachea and bronchi, was incapable of transmitting the disease to healthy sheep.

In the affected farms almost all varieties of veld were represented—grass, karoo, bushveld, and mixed veld—so it may be concluded that the character of the grazing exerts no influence on the incidence of the disease. Indirectly the quality of the grazing by affecting the condition of the animals is responsible for variations in the number of cases appearing in the flock. Animals in good condition do not contract the disease so readily under natural veld conditions, or it may be that the symptoms do not appear so soon as in sheep in poor condition. In the experiments no difference in susceptibility was noticed, and sheep in good condition which showed no symptoms during life were found on post-mortem to show well-defined pulmonary lesions.

Although no definite information is available, it follows from experimental evidence that the occurrence of the disease in a previously healthy flock follows either the introduction of infected animals or contact with a flock in which the disease exists. Owing to the fact that a kraal, in which infected animals have been kept for some time, retains the infection and is able to transmit the disease to healthy sheep, there is some risk of the disease being transmitted to a healthy flock which has gained access to an infected camping ground or kraal, but this factor may be eliminated under natural conditions. Kraaling of the sheep at night, by increasing the risk of infection by immediate contact, undoubtedly tends to increase the mortality in a flock. The disease has a very insidious course, and it may be transmitted from diseased to healthy animals before the symptoms become apparent. This is proved by the fact that some farmers who attempted to eradicate the disease in their flocks by immediate slaughter of all cases diagnosed, still found cases occurring even after this prophylactic measure had been carried out over a considerable period.

Cases of the disease usually occur singly, and no case is on record where an outbreak had occurred involving a large number of sheep in the same flock simultaneously. From this it can be deduced that the

contagion is not very virulent, and that animals under normal conditions in the veld possess a considerable power of resistance to the infection. The conditions, especially where the sheep are not kraaled, are such that an epidemic would not be expected.

The disease may also be spread to unfenced farms by infected sheep gaining access to the clean area or mingling with the sheep grazing thereon. Sheep purchased for breeding purposes may bring the disease on to a previously clean farm, and as the disease is difficult to detect in the early stages, this is a factor in the dissemination of the disease which must be carefully guarded against. One farmer reports a marked increase in the number of cases following the use of a crib for supplying salt to the animals. The cases decreased when the animals were returned to an area composed of "brak" veld, where salt was not administered. This observation, if correct, can only be explained by the fact that the use of the crib increased the possibility of infection by contact, or by considering the discharge from the nostrils, etc., infective and causing contamination of the crib.

Experimentally the bronchial discharges were not found to be capable of setting up the disease, even when inoculated into the trachea of healthy sheep, but as later experiments showed that kraals remained infective after the removal of the infected animals, it is quite feasible to suppose that a crib may also become a means of transmitting the disease, though what the actual infecting agent is must be left to future investigations to ascertain.

Symptoms—

The first symptom which is noticed is increased respiratory movements in excess of the normal, after the animal has been driven some distance. At this stage the animal may be in good condition and show no other symptoms. Later, a marked falling off in condition occurs. The animal becomes dull, and is generally found near the "tail-end" of the flock when the sheep are being moved. Marked respiratory distress is evident on exercise, the respirations being short and jerky. Symptoms of a catarrhal condition of the nasal mucosa appear. The muzzle and outer aspect of the nostrils are encrusted with dried discharges. The edges of the nostrils are wet with discharge, which may occasionally be seen dripping in clot-like masses from the nostrils. The nasal mucosa is injected and covered with a muco-catarrhal discharge. The catarrhal condition extends to the eyes. The eyelids become swollen and slight hyperaemia of the conjunctiva is apparent. The discharge mixed with the lachrymal fluid flows profusely over the cheeks. Coughing—particularly in the early morning—is always present at this stage. The cough is soft. The initial inspiration is short, and the expiration is prolonged, and is accompanied by moist *râles*. These *râles* can be easily detected in cases where there are marked lesions even during rest, and are a valuable aid to diagnosis. Later, the head is carried low and the animal becomes very debilitated. Great respiratory distress is present, and frequent fits of coughing occur, especially when the animal is driven. The mucous discharge from the nostrils becomes greater in quantity, and the animal is sometimes noticed to rub its nose on the ground during feeding. The appetite, which in the earlier stages was unimpaired, is now almost lost, and as a result of the debilitated condition the animal, when feeding, mostly progresses upon its knees. Anaemia, shown by the pallor of the skin and mucous membranes, is

present. Loss of patches of wool often occurs. Extreme emaciation ensues and the animal dies in a very debilitated condition. Intermittent attacks of diarrhœa may occur in the later stages, but this is not a constant symptom.

No pyrexia has ever been noted. The temperature in all cases under observation was normal or subnormal. As the disease is difficult to diagnose in the initial stages, it is not possible to form a definite opinion as to the duration of the disease. Cases sent to the Laboratory for experimental purposes have been kept under observation for varying periods up to twelve months. The diagnosis was in these cases confirmed on post-mortem. Under natural conditions in the veld death occurs very much earlier—usually at intervals varying from two to eight months. The life of the animal is undoubtedly prolonged if it is placed on a nutritious diet soon after the first symptoms become apparent. In animals so treated the disease seems to progress very slowly, and if the condition is kept up the sheep may appear to have recovered. Post-mortem examination of such animals, however, in most cases shows that there are extensive pulmonary lesions present which show no tendency towards localization or resolution.

Cases have been observed at post-mortem where the appearances presented would lead one to suppose that the disease had been arrested, and that an attempt at resolution was taking place. Such cases are, however, rare, and it is doubtful whether partial or complete resolution could occur, owing to the nature of the lesion in cases where the disease has progressed for some time. In such cases one is justified in supposing that complete recovery does not occur, and although the disease in such an animal may progress very slowly, if allowed to run its natural course it is invariably fatal.

Statistics from the infected area show an average mortality of 1.6 per cent. per annum. The number varies on different farms, and also varies in different years.

Differential Diagnosis—

The only disease prevalent in the affected districts which might be mistaken for jagziekte is the caseous lymphadenitis of Nocard. Differentiation in the early stages is difficult, as many animals may be the subject of both conditions. The greatly increased respiratory movements on exercise, and later the moist cough, nasal discharge, and bronchial *râles*, are diagnostic of jagziekte. Some farmers diagnose the condition by holding the suspected animals up by the hind legs, and noting if a discharge from the nostrils occurs. This method, although rather crude, is said to be of value in the diagnosis of early cases.

A condition which gives rise to clinical symptoms indistinguishable from jagziekte, is that produced by an extensive infection of the lungs with cysts of the *taenia echinococcus*. In one lot of sheep forwarded from Steynsburg to the Laboratory, an animal was found which during life showed symptoms which were similar to those of jagziekte. At the post-mortem, however, it was found that both lungs were badly infected with these cysts, which were of varying sizes, some as large as a goose egg. As a result the pulmonary tissue was by pressure very much atrophied, and the symptoms simulating jagziekte were due to the great reduction of respiratory area.

Pathological Anatomy: Macroscopic—

On opening the chest cavity, in an advanced case it is usual to find fibrous adhesions uniting the visceral pleura over the lesion to the costal pleura. A small quantity, up to 20 c.c. of fluid, may be present in the pleural sac. This fluid generally contains numerous flocculi, and either coagulates slowly to form a very weak clot, or does not coagulate at all. The appearance of the affected portion of the lung varies with age of the lesion. In the early stages the following appearance is presented:—The size of the lesion varies from the size of a haricot bean upwards—generally sub-pleural in position, and visible on the surface of the lung before section. The superimposed pleura is thickened and more opaque than that covering the normal pulmonary tissue, and may show small granulations, depending upon the age of the lesion. The lesion itself is easily distinguished from the surrounding lung tissue, but not sharply demarcated. It feels fleshy on manipulation, and projects the surface slightly when present immediately under the pleura. The lesion is hyperaemic and is surrounded by a hyperaemic zone extending into the normal pulmonary tissue. In the early stage one lobule only is affected, but later the lesion spreads to adjacent lobules. On closer examination of the lesion, with the overlying pleura still intact, it will be seen to be composed of a number of small greyish translucent nodules, each of which is about the size of a millet seed and surrounded by infiltrated lung tissue. These nodules can be detected on manipulation. On section, the cut surface of the section shows a similar appearance, the greyish nodules being distributed through the entire lesion and projecting from the surface of the section. On squeezing the lesion a small quantity of frothy mucus will be seen to exude from the cut surface, and from the smaller bronchi which have been incised. One or two such lesions may be found in the same lung, but the number rarely exceeds two.

In older lesions where a considerable area of the lung is affected (say, 2 inches or 4 inches in diameter), the centre of the lesion will be seen to have undergone fibroid changes. The overlying pleura is very much thickened and opaque, and often firmly adherent to the costal pleura. When this attachment is broken down or cut, it will be seen that instead of the surface of the lung being slightly projected, as was noted in the earlier lesion, a more or less marked depression of the surface is apparent, due to cicatricial contraction. This fibrosis diminishes towards the periphery of the lesion, where a condition resembling that seen in the earlier lesion is apparent.

The pleura overlying this portion of the lesion at the periphery has lost its glistening appearance, and appears rough and thickened, due to a commencing pleuritis. The lobulation has almost entirely disappeared; the lung tissue surrounding the lesion shows areas of atelectasis particularly towards the borders of the lung. Compensatory emphysema is also present, and is particularly marked in the anterior lobes. The centre of the lesion cuts hard, almost like tendon, and the section presents a whitish glistening surface. The openings of the smaller bronchi are almost entirely occluded, owing to connective tissue changes involving the mucous membrane, the bronchial tube, and peribronchial tissue.

Blood-vessels are difficult to detect, and the area is poorly supplied with blood. Towards the periphery the fibrosis decreases. The

bronchi with their very thickened walls are apparent, and may contain a mucoid plug. The surface of section is granular and corresponds to the appearance presented by the earlier lesion previously described. On compressing the lesion a granular frothy mucoid exudate mixed with blood is squeezed from the surface of section. On opening the larger bronchi, they will be found to contain a considerable quantity of granular frothy tenacious mucus. The mucous membrane shows well-marked thickening and diffuse hyperaemia. This condition is also present in the trachea, but to a much less degree. The mucous membrane lining the glottis is slightly thickened and oedematous, and often shows marked hyperaemia. The mucous membrane lining the air passages in the skull, particularly over the turbinated bones, are congested and covered with a catarrhal exudate.

Although the lesions are mostly confined to one lung, a number of post-mortem examinations made on advanced cases showed lesions in both lungs. In these cases it was evident from the appearance of the lesions that there was considerable difference in the ages, and it was apparent that the primary lesion had developed in one lung and later spread to the other. The area of the lung primarily affected was, in the majority of cases, found to be situated about the middle of the cardiac lobe of the right lung. This is probably due to the fact that a special bronchus is given off from the trachea a considerable distance above the bifurcation into the two main bronchial tubes, and this bronchus is distributed to the anterior and cardiac lobe of the right lung.

This, however, does not always hold good, as a number of cases on post-mortem showed primary lesions in the left lung. The cardiac lobe is usually the first portion to become affected, and later lesions may appear in the main lobe, or less frequently in the anterior lobe.

In a few cases post-mortem where this lobe was extensively affected, an appearance suggestive of a spread by contiguity was noted, a focus from which the lesion was spreading being found in that portion of the main lobe which abuts against the cardiac lobe. The surfaces of the pleura lining the cleft between the two lobes were firmly attached by fibrous adhesions.

Where considerable area of lung is affected, there is invariably a compensatory emphysema present in adjacent lung tissue. This is usually more marked in the anterior lobe, and varies in degree with the age and extent of the jagziekte lesion.

Associated with these changes described in connection with the respiratory apparatus, we also find well-marked alterations in the lymphatic glands in the bronchial and mediastinal regions. In cases where the disease has been present for some time, and well-marked changes are present in the lungs, these glands are found to be considerably enlarged and much softer to the touch than normal. The fatty tissue surrounding them is oedematous. On section the cut surface is usually of a pale slaty-grey colour, and there is a profuse discharge of lymph from the cut surface. These changes are confined to the glands of the thoracic cavity. No alterations could be found in the mesenteric glands or in any of the glands of the abdominal viscera, even where the organs themselves showed pathological changes.

The liver and kidneys usually show fatty changes of varying degree.

In the liver the changes in an advanced case of the disease are as follows:—The capsule is slightly wrinkled and the organ slightly

reduced in volume. The colour is lighter than that of normal liver, and the organ on section is more friable than is usually found. Examination of the cut surface shows that the peripheral zone of the lobule is paler than the centre.

The naked eye changes in the kidney consist in the presence of parallel lines of a pale grey colour in the cortex, extending towards the pelvis. The organ is not otherwise markedly altered. The following post-mortem reports of typical cases will give a good idea of the general post-mortem changes to be found in an advanced case of the disease:—

Description: Persian Sheep No. 6407.

Sex: Ewe.

Date of Death: 4th August, 1914.

Condition, fair. *Flesh* normal. *Fat* scarce.

Peritoneal Cavity: Normal.

Pleural Cavity: Empty.

Pericardium: 2 c.c. clear liquid.

Heart: Normal in size and consistence. Valves normal. Endocardium normal. Trabeculae of right ventricle enlarged. Anterior aorta normal. Foramen ovale closed. Coronary artery normal. *Thymus* small, with fat.

Tongue: Normal. *Palate* normal. *Pharynx* normal.

Retro-Pharyngeal Glands not enlarged. *Oesophagus* normal.

Larynx and Trachea: Mucous membrane, slight hyperaemia and injected. *Thyroid glands* normal.

Left Lung enlarged. Anterior, posterior, inferior, and superior lobes large patches with small amount of air. *Pleura* normal. On section tissue grey, transparent, elastic. Posterior part contains small calcified echinococcus cyst. Inferior lobe-tissue on section in some parts red, in other parts white, with a number of small foci 1 mm. in diameter, in other parts the foci are larger, 2 to 4 mm. In the anterior lobe are patches of reddish tissue, with some opaque or white nodules. Bronchi in patches, small, slightly enlarged. Bronchial mucous membrane normal. Caseous nodules $1\frac{1}{2}$ cm. in diameter.

Posterior Mediastinal Glands enlarged 2 to 3 cm. Consistence soft, white, with several caseous nodules $\frac{1}{2}$ to 1 cm. in diameter.

Right Lung: *Pleura* normal, only between posterior and middle lobe fibrous adhesions. *Middle Lobe* enlarged, hard; near apex of anterior lobe a small patch of atelectasis; the periphery also shows in parts atelectasis. Parts of lung emphysematous on section—pale, transparent, elastic. In the posterior lobe is a patch of hard tissue, reddish-grey, transparent. In the middle lobe on section the tissue is reddish-grey, friable, many small white or opaque nodules $\frac{1}{2}$ to 1 mm. in diameter scattered in small groups. In the atelectatic part of the anterior lobe the tissue is reddish-grey, with a number of groups of small opaque nodules. The bronchial mucous membrane is normal.

Bronchial Glands: Normal.

Spleen: Capsule smooth. Pulp reddish-brown. Consistence normal. Follicles and trabeculae distinct.

Suprarenal Glands: Normal. Traces of fat in cortex.

Kidney: Fat capsule well developed. Fibrous capsule is smooth. The organ is rich in blood, small opaque lines are seen in the cortex. Consistence normal. *Abdominal aorta* normal.

Duodenum: Normal. *Gall Bladder*: Clear yellow bile, mucous membrane normal.

Pancreas: Normal.

Liver: Serosa normal, tissue brown, acini small, fairly transparent. Capsule not thickened. Consistence normal. Periportal glands not enlarged.

Bladder: Empty mucous membrane normal. *Ovary* normal. *Uterus* contains a little brown mucus, the cotyledons are small and of a brownish colour. *Vagina* normal. Near vagina small cyst 2 cm. diameter with clear contents.

Stomach: Normal. *Intestines*: Few parasitic nodules, mucous membrane normal. Follicles small.

Pathological Anatomical Diagnosis.—Chronic induration of lungs. Caseous lymphadenitis. Circumscribed emphysema of lungs. Fatty degeneration of liver and kidneys. Uterus after abortion. Hypertrophy of right ventricle.

Description: Cross-bred African, No. 6482.

Sex: Ewe. *Age*: Very old.

P.M. Number: 9009.

Date of Death: 4th August, 1914.

Condition: Fair. Fibrous attachments on pleura covering left lung at upper extremity of second rib (1 inch by 1½ inch). Catarrhal discharge on upper lips and nostrils.

Right Lung: Anterior lobe, extensive lesions, surface of lung mottled in appearance, with numerous translucent centres. On palpation numerous nodules and masses of lung affected with pneumonia. On section numerous translucent areas, size of pea, with some unaffected lung tissue interspersed.

Cardiac Lobe: On section fleshy, similar lesions to anterior lobe.

Main Lobe: Anterior portion numerous discrete nodules, pearly-grey in colour, and surrounded by hyperaemic zone.

Left Lung: Anterior lobe considerably atrophied and affected with old jagzietke lesion. Lung tissue firm and fleshy, and not collapsed. *Cardiac Lobe*: Consistence fleshy, cutting firm on section; catarrhal discharge from surface of section; anterior portion of main lobe numerous lesions varying in size from a pea to walnut. Lesions corresponding to that found in other lung.

Trachea: Some foam and catarrhal exudate. Vessels injected.

Pericard: Normal. *Epicard*: Normal. Endocard of both left and right ventricles normal.

Bronchial and Mediastinal Glands: Slightly enlarged.

Liver: Lobulations distinct. Parenchyma normal.

Gall Bladder: Distended with normal bile.

Spleen: 11 by 7½ inches, pulpa soft, malpighian bodies distinct.

Kidneys: Normal. *Uterus*: Gravid, about two-month-old foetus.

Abomasum: Few *Strongylus contortus*, mucosa normal, other stomachs normal. *Caecum*: Normal. *Cyst. tenuicollis* attached to small intestines. Few *Oesophagostomum columbianum*. Numerous nodules present in ileum. *Jejunum*: Nodules rare, mucosa normal.

Rectum: Normal.

Supernares: Normal. Mucosa covering turbinated bone of both nostrils darkly injected.

Septum nasi: Similar condition.

Pathological Diagnosis.—Chronic catarrhal pneumonia—Jag-ziekte.

Microscopic Lesions in Jag-ziekte.

(1) *Changes in the Pleura*—

On examination of the pleura over the lesions, it will be seen that the differentiation into serous and connective tissue layers can no longer be made out. The pleura is very much thickened, and is composed of organized connective tissue with the fibres arranged at right angles to the lung surface, and presenting a ragged free border, due to the varying lengths of the connective tissue strands occurring as a result of rupture of pulmonary with costal pleura. The sub-pleural connective tissue layer shows a well-marked round-celled infiltration, with an increase in connective tissue elements. In the earlier stages the condition shown is that of a fibrinous deposit in the pleura, which persists for a considerable time, and later becomes transformed by a process of organization into fibrous connective tissue.

(2) *Changes in the Parenchyma*—

In most of the sections examined it was noted that in those showing commencing pneumonic lesions, there was invariably present a local acute congestion. The interalveolar capillaries were filled with blood-cells and enlarged, and in many cases extravasations had occurred into the alveoli, which were filled with erythrocytes.

The most obvious lesions, however, which will be observed on microscopical examination of the affected portion of the lung, are in the sections through the nodules already noted in the macroscopic examination. These nodules are probably of lymphoid origin. They are disseminated through the affected portion of the lung parenchyma, occurring chiefly in connection with the bronchi, but also found isolated in the alveolar network. They are spherical or oval in outline, and although well differentiated, the line of demarcation is not sharp. In most cases an attempt at encapsuling can be made out.

The size of the nodule varies from .2 to .4 millimetres.

The centre of the nodule consists of an open network of cells of two types—first, cells with large nuclei (resembling plasma cells), rounded or rhomboidal in shape and varying in size from that of a leucocyte to twice its diameter. The nucleus is of varying shape, and in some cases eccentric in position. The intranuclear network is markedly open, and contains a few darkly staining chromatin bodies. In some cases the intranuclear network is not so apparent, or the nucleus appears as a clear space with occasional chromatic bodies disseminated through it, or arranged at the periphery.

The second type consist of small cells resembling fibroblasts; cells with rounded compact nuclei surrounded by a very thin halo of protoplasm, and resembling a mononuclear leucocyte. Many of the nuclei of both these types of cells may be seen to be in varying stages of karyokinetic division.

There is an absence of fibrous stroma in the centre of the nodule, and giant cells have never been found.

This central zone is surrounded by a narrow zone composed of fibroblasts, and young connective tissue fibres arranged in concentric layers, and giving the appearance of an attempt to encapsule the nodule. To the outer side of this zone is an area of varying width,

composed of small round cells densely packed and supported by a fair quantity of fibrous stroma. The margin is not so sharply defined, and extends into the interlobular connective tissue of the surrounding pulmonary parenchyma. These nodules are usually discrete, and either surrounded by alveolar tissue or intimately associated with a bronchus. At a later stage the nodule increases very much in size, the appearance of encapsulating disappears, and an invasion of the lung tissue follows. The round cells will be seen to spread along the interlobular connective tissue spaces, obliterating the alveoli, and, later, giving an appearance to the section resembling a lympho-sarcoma, with here and there a bronchus, this being the only indication of the original tissue. The smaller bronchi show changes resulting from the chronic catarrhal pneumonia present. The mucous lining is very much thickened, and composed of several layers of cells which are irregular in outline. Sections of the bronchi in the affected tissue often show projecting ridges of mucous membrane which are seen to be polypoid in form. Under a high magnification the base of these projections will be seen to contain cross-sections of muscle bands which appear hypertrophied, and are probably derived originally from the circular muscular coat of the bronchus which has, in such cases, almost entirely disappeared.

In some of the sections examined, a condition was noted in which it would appear that proliferation of the bronchi had taken place. Groups of small bronchial tubes could be seen cut across, each having a thin fibrous wall and lining of mucous membrane such as is present in a small bronchus. This appearance may, however, be due to the section of the very much hypertrophied mucous membrane of a bronchus where the line of section passes through the corrugated mucosa. No trace of a bronchial wall could, however, be seen around the groups of small bronchi referred to, so it is possible that in some cases actual proliferation of bronchi does occur.

The lumen of the bronchi contains either a solid mucous plug, or a quantity of exudate composed of catarrhal cells. The walls of the bronchi in their outermost covering are tremendously increased in thickness, due to the infiltration of the peribronchial tissue with numerous small round cells. This, together with the hypertrophy of the mucous membrane, results in some cases in partial occlusion of the bronchus. Total occlusion may then follow by the formation of a mucous plug which fills up the lumen.

Similar infiltrations may be observed occasionally in the perivascular coats of branches of the pulmonary artery, particularly where these are associated with an affected bronchus.

The alveolar tissue in the neighbourhood of lesions shows changes due to a lobular pneumonia, thickening and infiltration of the interalveolar and interlobular connective tissue, and an exudate of catarrhal cells occupying loosely the cavity of the alveoli. As in other pneumonias of this type, there is an absence of fibrin in the alveolar contents.

In the older parts of the lesion it will be found that the cellular elements have to a large extent disappeared. The alveoli have become obliterated, and the true lung tissue is replaced by tissue which has resulted from the activity of the fibroblasts. The section resembles scar tissue, and is composed chiefly of white fibrous tissue bundles, with here and there a few cellular elements. It is difficult to say what tissue the primary lesion arises in connection with, but from the examination of a number of specimens of lung in which only very

limited lesions were present, I have come to the conclusion that the peribronchial tissue of the smaller bronchi is the first tissue affected.

Earlier in this paper the view was expressed that inhalation was probably the means of infection. If this is correct, and it would appear to be so from the experimental evidence, then we could expect to find the primary lesion in connection with the lymphatic spaces of the peribronchial connective tissue.

In order to ascertain to what extent the lymphoid nodules were associated with the disease, as well as to obtain more information regarding the pathological changes, a histological examination was carried out on the lungs of fifteen sheep which had been used for experimental work, the greater number of which showed well-defined macroscopic lesions of jagziekte on post-mortem. A précis of the findings at the microscopic examination of sections of the lung lesions of these animals is given herewith.

Case 1. Sheep No. 5354.

Emphysema and congestion were not marked. The bronchi showed chronic bronchitis with well-marked swelling and desquamation of the mucous membrane. The walls of the bronchi showed infiltration. The alveoli were in parts obliterated by the extensive interstitial cellular proliferation. Marked thickening of the arterial walls was present. Lymphoid nodules were present in great numbers in various parts of the field.

These were developed in connection with all the tissues of the lung, and were of varying sizes, some sharply circumscribed and others apparently infiltrating. Proliferation of the bronchi was also present.

Case 2. Sheep No. 6407.

Emphysema and congestion were not marked; chronic bronchitis was present, with thickening of the epithelial lining of the bronchi and some desquamation. The walls of the bronchi showed marked thickening and round-celled infiltration. The alveoli were mostly empty, and the interalveolar tissue thickened by a round-celled infiltration. The walls of the arteries were thickened, and dilatation of the capillaries and veins was present. Numerous lymphoid nodules were present in various parts of the section, circumscribed and infiltrating. Proliferation of the bronchi was not marked.

Case 3. Sheep No. 6479.

Very acute congestion was present. The mucous membrane lining the bronchi was thickened, but otherwise no marked changes were present. The peribronchial tissue was infiltrated with round cells. The alveoli were mostly empty and circular, and the interalveolar connective tissue infiltrated and thickened. Numerous haemorrhages were present in parts of the field. Lymphoid nodules were not frequent, and developed only in connection with the bronchi. No proliferation of the bronchi was present.

Case 4. Sheep No. 6480.

Emphysema was well marked, and the section in some parts of the field showed well-marked congestion. Chronic bronchitis was present, with thickening of the mucosa and considerable desquamation. The bronchi contained a quantity of catarrhal products, and the walls were very much infiltrated with round cells. The interalveolar tissue was

infiltrated with round cells. The arterial walls were thickened, and the veins dilated and full of blood. Haemorrhages were present in many parts of the field.

Numerous lymphoid nodules were present, of varying sizes and developed in connection with all the pulmonary tissue, some sharply circumscribed and others infiltrating. No proliferation of bronchi could be detected.

Case 5. Sheep No. 6482.

Marked emphysema was present, but congestion was absent. Slight bronchitis was noted, shown by thickening of the bronchial mucosa. Infiltration of the peribronchial tissue was present, and the alveoli were for the most part empty, and the interalveolar tissue very much infiltrated and thickened. Thickening of the coats of the arteries was present; there was also considerable dilatation of the veins and capillaries. Haemorrhages were rare. Lymphoid nodules were very numerous and of varying sizes. Proliferation of the bronchi was well marked; in some places groups of about twenty bronchi could be seen severed.

Case 6. Sheep No. 6483.

Emphysema was present in places, and slight congestion was also apparent. There was chronic bronchitis, with well-marked round-celled infiltration of the peribronchial connective tissue; the contents of the alveoli were cellular, but the alveoli were in most cases empty. The interalveolar tissue was not markedly infiltrated, nor was much thickening of the walls of the arteries noted. Haemorrhages were not seen. Numerous lymphoid nodules were present, disseminated in the parenchyma, and also in connection with the bronchi. Proliferation of the bronchi was not marked.

Case 7. Sheep No. 6484.

Very marked emphysema was present. The changes in the bronchial walls were those of chronic bronchitis. The walls and peribronchial tissue were very much infiltrated. The alveoli were for the most part filled with catarrhal products, and the interalveolar tissue was infiltrated with round cells, and very much increased in thickness. Thickening of the walls of the veins and arteries was present, and dilatation of the capillaries could be seen. No haemorrhages were seen. Lymphoid nodules were numerous, and scattered diffusely through the section. Proliferation of the bronchi was not apparent.

Case 8. Sheep No. 6485.

Emphysema was well marked. There was no congestion. The bronchial walls were very much thickened, and desquamation of the epithelial lining was noted. Infiltration of the peribronchial tissue was well shown. No changes were noted in the walls of the arteries, and the veins and capillaries were normal. Lymphoid nodules could not be easily made out. Condition is that of a commencing pneumonia, and shows the thickening of the peribronchial tissue well marked, with round-celled infiltration extending into the surrounding tissue. The section is not typical of jagziekte.

Case 9. Sheep No. 6486.

The section in this case showed a well-marked pleuritis with

ateleactasis of the sub-pleural tissue. No congestion was seen. A chronic bronchitis was present, the mucous membrane lining the affected bronchi being swollen and desquamating. Infiltration of the peribronchial tissue was very marked, being in some places five times its normal thickness. Surrounding tissue showed no trace of its original structure, and was replaced by partly formed connective tissue fibres. In some parts of the field the alveoli were filled with cellular contents of a catarrhal nature. The walls of the arteries were in most parts thickened, but dilatation of the capillaries was not marked. No haemorrhages were noted. Numerous lymphoid nodules of varying sizes were present in the field examined, being principally associated with the bronchi. Proliferation of the bronchi was well marked.

Case 10. Sheep No. 6486a.

Marked emphysema was present, but no congestion. The changes in the bronchi were those of a chronic bronchitis, with a marked rounded infiltration extending into the surrounding peribronchial tissue. In the greater part of the section the alveoli were normal. The arterial walls were unaltered. The capillaries and veins were normal. No haemorrhages were seen. Lymphoid nodules were rare, small in size, and associated only with the bronchi. This section showed a very early stage in the disease, and it was very easy, from an examination of a few fields, to follow the progress of the pneumonia from the first tissue affected to a condition of general carnification.

Case 11. Sheep No. 6489.

Emphysema was present, but not marked. Slight congestion was observed in parts of the field. Chronic bronchitis was present, with well-marked thickening of the bronchial walls, and infiltration of the peribronchial tissue. The contents of the alveoli were cellular. The interalveolar tissue was very much infiltrated with round cells. Changes in the arteries and veins were not well marked. No haemorrhages were seen. Lymphoid nodules were numerous, of varying sizes, and, in many cases, showed signs of infiltrating the surrounding tissue. These nodules were found in connection with all the lung tissues. Proliferation of the bronchi was present, but not well marked.

Case 12. Sheep No. 510.

Slight emphysema and congestion were present. Changes in the bronchi were very slight. Slight infiltration of the peribronchial tissue was apparent. The alveoli were filled with catarrhal products, and, in many parts of the section, interstitial cellular proliferation was well marked. Lymphoid nodules were rare, and developed only in connection with the bronchi. Proliferation of the bronchi was well shown.

Case 13. Sheep No. 5353.

Marked emphysema was present, but no congestion. The bronchi showed the usual changes of chronic broncho-pneumonia. The bronchi were in many cases filled with a mucous plug. Marked infiltration of the peribronchial tissue was present. The original cellular network of the lung could not in many places be made out, the lung tissue being completely carnified. Marked thickening of the arterial walls was present, and also dilatation of the veins and capillaries. Numerous haemorrhages were present. Lymphoid nodules of varying sizes were present, circumscribed and infiltrating, and scattered diffusely throughout the lung tissue. Proliferation of the bronchi was very marked.

Case 14. Sheep No. 5349.

Slight congestion was present, but no emphysema. Changes due to chronic bronchitis were present in the bronchi. Marked infiltration of the peribronchial tissue was present. The contents of the alveoli were cellular, and the interalveolar tissue was marked and infiltrated with round cells. Thickening of the arterial walls was marked, but there were no pathological changes in the veins or capillaries. No haemorrhages were present. Lymphoid nodules were not frequent, small in size, and showed evident signs of active proliferation, and in some parts showed marked symptoms of infiltration. This section showed well the commencing lesion of jagziekte.

Case 15. Sheep No. 5346.

No marked lesions of congestion or emphysema were present. There was chronic bronchitis with thickening of the bronchial wall, and the mucous membrane present. The bronchi were in many cases filled with a mucoid plug. The contents of the alveoli were catarrhal, and in some parts of the field the contour of the alveoli could no longer be seen, owing to connective tissue changes. Marked thickening of the arterial walls and dilatation of the capillaries were present. No haemorrhages were noted. Lymphoid nodules were present of varying sizes, and showing infiltration of the surrounding tissue. A few smaller nodules were sharply circumscribed, showing no tendency to infiltrate. Proliferation of the bronchi was very well marked.

To sum up, therefore, we have in jagziekte a pneumonia of a chronic catarrhal nature, which progresses in the affected lung by continuity and contiguity, and is characterized by the presence of nodules in the pneumonic area of a lymphoid nature, which show a marked tendency to infiltrate the lung tissue immediately surrounding them. These nodules may develop in connection with the bronchi, or in the interalveolar connective tissue. The other changes in the lung tissue are those of a broncho-pneumonia, viz., exudation of catarrhal products into the alveoli, interstitial fibroid changes, bronchitis and peri-bronchitis, and thickening of the coats of the arteries. As a result of these changes a general fibrosis of the affected part of the lung ensues, giving rise to a carnification of the tissues, and later fibrotic changes which render the lesion hard and dense, and almost cartilaginous in consistency. As a result, alterations of the healthy lung tissue occur at some distance from the lesion, viz., emphysema, in the anterior lobes, and towards the edges of the lung atelectasis; pleuritis, of a degree varying with the age and extent of the lesion, is constantly present, and is intimately associated with the lesion.

In the post-mortem examinations made, it was noted that the disease was in some cases complicated by the presence of abscess formation in the lungs and lymphatic glands connected therewith, due to the presence of the Nocard-Prieze bacillus. In many parts of South Africa this is a very common condition, but usually does not give rise to any symptoms during life, except where the infection is very extensive. When complicated with jagziekte, however, it has been noted that there is a greater tendency to breaking down of the pulmonary abscesses, and the formation of caverns containing inspissated pus, probably derived from the original abscess, mixed with a considerable quantity of brownish-red semi-fluid material. In no case which came under my notice was any tendency to breaking down

observed in jagzikte lesions, except where such lesions were complicated with the lesions of caseous lymphadenitis.

Small calcareous nodules of parasitic origin are often found in lungs of sheep on post-mortem. When associated with jagzikte lesions, these nodule do not differ in any marked degree from those found in normal lung, nor do they appear to cause any alterations in the lesion.

Period of Incubation—

Under natural conditions this is very difficult to determine, on account of the difficulty in detecting early cases. Well-marked pulmonary lesions are always present in animals which show clinical symptoms. Healthy sheep placed in close contact with infected animals, and slaughtered after a period of eight days, have shown well-marked lesions on post-mortem examination. As the condition in these cases undoubtedly had existed for a few days, I think I am justified in stating that, under experimental conditions, lesions may be found in incontacts in from three to five days. In this connection, however, the rapid course of the disease in animals kept in close confinement, which was noted in the experimental work, must be taken into consideration.

Treatment.—

Various local remedies have been used against this disease, but farmers are agreed that, while a plentiful diet prolongs the life of the animals affected, it is impossible to cure the animals. Many forms of medicinal treatment have been tried, but without success.

Prophylaxis—

Eradication of the disease from the infected farms can only be carried out successfully by the most stringent prophylactic measure. Many progressive farmers who recognize the infectious nature of the disease take the precaution to slaughter all animals suspected of being affected, and confirm their diagnosis by post-mortem examination. This is the only effective measure of stopping the further spread of the disease which is at present known. Unfortunately, even under such stringent measures, cases continue to occur, due to incipient cases in the flock in which clinical lesions are not yet apparent. Segregation of all sheep which show suspicious symptoms should be practised on farms where the disease is known to occur, and all cases in which the disease is recognized should be immediately slaughtered.

Kraals in which sheep are kept during the night should be as roomy as possible, or, better still, the animals should be placed at night in a fenced camp; thereby the danger of transmission by immediate contact is considerably lessened. Troughs in which salt, etc., is given to the sheep should occasionally be cleaned out.

As importation of sheep is a fruitful source of spread, all sheep brought from another area should be segregated, and kept for at least a month under observation, before being allowed to mix with the flock. Fencing of the farm to prevent contact with neighbouring flocks is essential, if the disease is to be kept from gaining access to the farm.

The flesh of the affected animals is usually utilized as rations for the Kaffirs. This procedure would not appear to influence the further spread in any way, as experimentally the tissue failed to reproduce

the disease either by inoculation or drenching, and so it is unlikely that bones and other refuse remaining near the kraals would be a source of infection.

DESCRIPTION OF PLATES.

- I.—Section through the pleura of affected lung showing pleuritis.
 - II.—Section through a young nodule associated with a bronchus, showing round-celled infiltration of peribronchial connective tissue, thickening of the bronchial mucous membrane, and a catarrhal pneumonia in the adjacent lung tissue.
 - III.—Nodules in the parenchyma, with well-marked round-celled infiltration in the interalveolar connective tissue in the vicinity.
 - IV.—Similar to No. III.
 - V.—Transverse section of bronchus, showing hypertrophy of the folds of the mucous membrane, forming outgrowths into the lumen.
 - VI.—Section of affected lung showing broncho-pneumonia.
 - VII.—} Sections through a young nodule. Lung tissue in vicinity shows
 - VIII.—} interstitial pneumonia.
- (Sections I to VI, Zeiss Objective, 16 mm.; Ocular 4.)
 (Sections VII and VIII, Zeiss Objective, 8 mm.; Ocular 4.)

PART II.—EXPERIMENTAL WORK.

IN March, 1912, a series of experiments were commenced by Sir Arnold Theiler, Director of Veterinary Research, in order to elucidate the nature of the infection in this disease.

Owing to his departure for Europe on leave in September, the experiments were temporarily discontinued, and were recommenced by me on my return to the Laboratory in January, 1913.

The healthy animals used in the experiment were mostly aged Merino hamels. A considerable number of Persian cross-breds were also used. The sheep were purchased from an area where jagziekte had never been found, and, previous to the experiments in connection with jagziekte, had been used at the Laboratory for the production of blue-tongue vaccine.

A definite diagnosis was made in practically all cases, as the animals were kept under observation until such time as slaughter became necessary in connection with this or other experiments; no cases have ever been noted in other sheep at the Laboratory, so it can be concluded that this area is free from the disease.

Infected animals were supplied to the Division by Mr. Bekker, Steyusburg, to whom best thanks are due for the assistance thus rendered during the investigations.

These initial experiments consisted of attempts to transmit the disease to healthy sheep by the administration or inoculation of material taken from affected animals. The immediate results of the experiments were apparently negative, but on slaughtering some of the inoculated animals early in 1913, I found that one of these sheep had well-marked lesions of jagziekte. It was therefore decided to repeat the experimental work done, and to continue the inoculation experiments with other infected materials, in order to arrive at a

definite conclusion as to whether the disease was an inoculable one or not.

With this in view the following experiments were undertaken, the list including also the original experiments carried out by Sir Arnold Theiler.

Experiment I.

To ascertain if an emulsion of lymphatic glands from the neighbourhood of the lesion produced the disease when drenched.

Three sheep were drenched 6/7/12 with an emulsion of glands of sheep 4163, and kept under observation until 9/4/13.

Result, negative.

Experiment II.

To ascertain if the disease could be transmitted by the injection of blood of infected animals.

(a) Three sheep were injected subcutaneously with from 10 to 50 c.c. blood, and kept under observation for periods varying from two to six months.

Result, negative.

(b) Nine sheep were given intravenous injections of blood, quantities varying from 10 to 150 c.c., and kept under observation from six to eight months.

Result.—One animal (No. 1827) which received 20 c.c. blood on 25/7/12, was found to be suffering from jagziekte when a post-mortem was made eight months later.

Experiment III.

To ascertain if the disease could be transmitted by bronchial exudate.

(a) Two sheep were drenched with 4 oz. each of bronchial exudate on 21/1/13, and kept under observation for six months.

Result.—One animal showed a temperature reaction from 7/4/13 to 19/4/13, but no lesions of jagziekte could be demonstrated in either on post-mortem.

(b) Ten sheep were inoculated into the trachea with quantities of bronchial exudate varying from 2 to 5 c.c., and kept under observation from three to thirteen months.

Result.—One animal (1827) which had been inoculated with 5 c.c. of bronchial exudate was found to be affected with jagziekte on post-mortem.

(c) Five sheep were injected into the jugular vein with quantities of bronchial exudate varying from 2 to 9 c.c. Animals were under observation from one to eighteen months.

Result, negative.

(d) Two sheep received a subcutaneous injection of 10 c.c. of bronchial exudate, and were under observation for three and six months respectively.

Result, negative.

(e) Six sheep were injected with from 2 to 5 c.c. of bronchial exudate into the lung parenchyma, and kept one to fifteen months under observation.

Result.—One animal (1827), which received 3 c.c. on 27/10/12, was found to be affected with jagziekte on post-mortem.

Experiment IV.

To ascertain if the disease could be produced by administration or inoculation of the affected portions of lungs of diseased sheep.

(a) Eight sheep were drenched with quantities of emulsion of lungs in saline, varying from 20 to 300 c.c. The animals were from two to fifteen months under observation.

Result, negative.

(b) Five sheep were inoculated into the jugular vein with from 2 to 100 c.c. of emulsion of affected lung in saline, and kept under observation for periods varying from three to five months.

Result, negative.

(c) Eight sheep received a subcutaneous inoculation of from 5 to 20 c.c. of emulsion of affected lung, and were kept under observation for varying periods up to six months.

Result, negative.

(d) Seven sheep received intratracheal injections of emulsion of lung in quantities varying from 2.5 to 10 c.c., and were kept under observation from one to nine months.

Result, negative.

(e) Sixteen sheep were injected into the lung parenchyma with quantities of emulsion of lung from 5 to 60 c.c., and kept under observation for periods up to three and a half months.

Result, negative.

(f) One sheep received 10 c.c. of emulsion of affected lung intraperitoneally, and was under observation for over two months.

Result, negative.

(g) Two sheep were injected into the caput muscles with 10 and 20 c.c. respectively of lung emulsion, and were under observation for one and two weeks.

Result, negative.

Experiment V.

To ascertain if the disease could be produced by drenching with faeces.

(a) Six sheep received doses of faeces up to 500 c.c., and were kept under observation for two to twelve months.

Result, negative.

Résumé.

As a result of the above experiments only one animal (1827) contracted the disease. This sheep was used in three of the experiments, and as none of the other sheep subsequently inoculated with similar materials contracted the disease, it was concluded that the infection in this case must have been accidental, and in no way associated with the inoculations. Transmission by contact with an infected animal was suspected, and to ascertain if this was the case a further series of experiments were undertaken as follows:—

Experiment VI.

To ascertain if infected sheep, when placed in contact with healthy animals, were capable of transmitting the infection, and, if so, in what time lesions of jagziekte became evident.

For this purpose ten sheep were taken which came from an infected area, all of which were suspected to be suffering from the disease. These animals were distributed into four loose boxes, and into three of these boxes five healthy sheep were also placed, six healthy sheep being placed in the remaining box. The boxes were not cleaned out on three successive occasions, at intervals of two, eight, and ten means of rack and troughs, and the conditions were such as to effect the greatest risk of transmission of the infection.

Slaughter of some of the incontacts from each box was carried out on three successive occasions, at intervals of two, eight, and ten days, and a careful post-mortem examination made in each case. Definite and well-marked lesions were found among the new incontacts in three animals—one in each of three different loose boxes. In the remaining loose box none of the incontacts were found to be affected. The diagnosis was in all cases confirmed by a micro-pathological examination. No animals were found affected in the first batch of sheep which had been two days in contact. In the animals which had been eight days in contact, two were found to have lesions in the left lungs. In one of the animals the affected area was about the size of a walnut. Slaughter of the remaining animals was carried out on the tenth day, and among these one animal on post-mortem examination was found to be affected.

A lesion the size of a hazel nut was present in the anterior lobe of the left lung.

Conclusions.

1. The disease is capable of being transmitted from infected to healthy sheep by immediate contact.
2. Definite lesions of fairly large size have been demonstrated after a period of eight days, and it is possible that macroscopic lesions could be detected after a period of about five days in contact.
3. The absence of the disease among the incontacts in one of the loose boxes, may have been due to the absence of the disease among the supposed infected animals, either completely or in a form in which transmission was possible in the longest period chosen, viz., ten days. In this box there were no sheep clinically affected among the suspects, whereas in each of the other three boxes, one of the suspected animals showed clinical symptoms of the disease.

Experiment VII.

Twelve healthy sheep, which had lost their ear numbers, were placed in a loose box. A few days later, by accident, two infected sheep were placed in the same box. When this condition was discovered, it was decided to allow all the animals to remain.

At the commencement of the last experiment, these animals had been three months in contact, so it was decided to confirm the results of this experiment by slaughter, and subsequent post-mortem examination of these twelve "no number" sheep, for signs of infection. This was carried out, and as a result of the examination, it was found that six out of the twelve showed definite lesions of jagziekte.

This result confirmed beyond the possibility of doubt that the disease was capable of being transmitted by immediate contact.

Experiment VIII.

To ascertain if a loose box, in which infected animals had been kept for some time, was capable of conveying the infection to healthy sheep.

For this experiment three of the loose boxes in which cases of jagziekte had occurred in the previous experiment were used. The bedding, etc., was allowed to accumulate during this experiment, so as to increase the risk of transmission as much as possible.

The ten infected sheep were taken which had been used in the last experiment, and placed in two boxes—five animals in each box. Care was taken to have clinically affected sheep in each box.

No infected animals were placed in the third loose box.

Eight healthy animals were placed in each box.

Post-mortem examinations were carried out on incontacts from each box at intervals of ten, twenty, and thirty days.

Results.—In the loose boxes containing the healthy and infected animals, two sheep were found to have contracted the disease. Both of these cases were found in the same box. The first case was observed in a sheep which had been ten days in contact. Post-mortem examination revealed the presence of a lesion, about the size of a pea, under the pleura of the right cardiac lobe, and the diagnosis of jagziekte was confirmed by microscopic examination.

The second case of the disease was found in an animal which had been thirty days in contact. Extensive lesions of jagziekte were present in the right lung of this animal.

Among the healthy sheep placed in the infected loose box, one case occurred in a sheep which had been in contact with the loose box for a period of thirty days. Definite and extensive lesions of jagziekte were present. No other case was found among the animals in this loose box.

Conclusion.

1. The occurrence of one case among previously healthy animals placed in an infected box proves that the contagion is fixed, and persists in a form capable of communicating the disease for some time after infected animals are removed.

2. The results in healthy sheep in contact with infected animals further confirm the two previous experiments.

General Remarks.

From the foregoing experiments, it will be seen that the conditions of transmission point to a specific virus being the cause. It was at first thought that the disease might be of parasitic origin, but this theory had to be laid aside, as no parasites were ever found associated with the pulmonary lesions, although careful search was made both in the lesion and in the bronchial exudate.

The absence of a causal organism in smears made from the lesions or in cultures, does not necessarily mean that such does not exist, and further experimental work will be necessary in order that more data may be obtained regarding the conditions governing transmission and, if possible, to ascertain what is the actual causal agent.

The experiments were discontinued in September, 1913, owing to my departure on leave, but I hope to be able at some future time to resume the study of the disease.

1.—*Emulsion of Glands.*

No. of Sheep.	Sex.	Age.	Date of Experiment.	Origin of Material.	Method.	Quantity.	Date of Death.	Remarks.
A. 3853	Hamel	Aged	6/7/12	Mediastinal and bronchial glands, S.4163	Drenched	?	9/4/13	Killed for post-mortem.
3852	"	"	6/7/12	Mediastinal and bronchial glands, S.4163	"	?	9/4/13	Killed for post-mortem; used in experiment 4 D.

2.—*Blood.*

A. 4940	Hamel	Aged	21/1/13	Blood, S.4681	Subcutaneous	10 c.c.	7/7/13	Killed for post-mortem.
5062	"	3 years	22/5/13	" S.5300	"	50 c.c.	8/7/13	" "
4890	"	Aged	22/5/13	" S.5300	"	50 c.c.	8/7/13	" "
B. 2187	Ewe	3 years	1/7/12	Blood, S.4161	Intrajugular	20 c.c.	28/1/13	Killed for post-mortem; used in experiments 3 E and 3 B.
3367	—	—	1/7/12	" S.4162	"	5 c.c.	—	Discontinued observations in experiment 3 B.
1827	Ewe	3 years	1/7/12	" S.4161	"	20 c.c.	18/3/13	Killed for post-mortem; used in experiments 3 B and 3 E.
3322	—	—	15/7/12	" S.4162	"	20 c.c.	11/4/13	Killed for post-mortem; used in experiments 3 B and 3 F.
3371	—	—	15/7/12	" S.4162	"	20 c.c.	14/8/12	Killed for post-mortem; used in experiment 3 B.
4749	Hamel	3 years	21/11/12	" S.3840	"	15 c.c.	1/4/13	Killed for post-mortem.
4941	"	Aged	21/11/12	" S.4681	"	10 c.c.	7/7/13	" "
1833	"	3 years	21/11/12	" S.4161	"	20 c.c.	7/2/13	Killed for post-mortem; used in experiments 4 E and 3 B.
3779	"	Aged	12/2/13	" S.3950	"	150 c.c.	7/7/13	Killed for post-mortem.

3.—*Bronchial Mucus.*

No. of Sheep.	Sex.	Age.	Date of Experiment.	Origin of Material.	Method.	Quantity.	Date of Death.	Remarks.
A. 4539 4730	Hamel "	Aged 3 years	21/1/13 21/1/13	Bronchial mucus, S.4681 "	Drenched "	4 oz. 4 oz.	7/7/13 22/4/13	Killed for post-mortem. Killed for post-mortem; temporary reaction 7/4/13 to 19/4/13.
B. 1833 1827 3322	Ewe " —	3 years " —	— — —	Bronchial mucus, — " " "	Intratracheal " " "	5 c.c. 5 c.c. 5 c.c.	7/2/13 18/3/13 11/4/13	Killed for post-mortem; used in experiments 4 E, 2 B. Killed for post-mortem; used in experiments 2 B, 3 E. Killed for post-mortem; used in experiments 3 F, 2 B.
3853 3371 3367	Hamel — —	Aged — —	7/2/12 5/5/12 5/5/12	" " " " " "	" " " " " "	3 c.c. 5 c.c. 5 c.c.	9/4/13 14/8/12 —	{ Killed for post-mortem; used in experiment 1 A. Killed for post-mortem; used in experiment 2 B. Discontinued observations 22/11/12; used in experiment 2 B.
2187 4733 4702 3833	Ewe Hamel " "	— Aged " "	5/5/12 21/1/13 21/1/13 7/3/13	" " " " " "	" " " " " "	5 c.c. 5 c.c. 5 c.c. 2 c.c.	28/1/13 7/7/13 2/4/13 —	Killed for post-mortem; used in experiments 3 E, 2 B. Killed for post-mortem. { Discontinued observations 22/11/12; used in experiment 2 B.
C. 3836 3823 4945 4742	Hamel " " "	Aged " " "	7/3/12 7/3/12 21/1/13 21/1/13	Bronchial mucus, { S.3379 S.3830 } " " " "	Intrajugular " " "	2 c.c. 5 c.c. 5 c.c. 5 c.c.	— 7/7/13 22/2/13 7/7/13	Discontinued 20/1/12. Killed for post-mortem. Died. Killed for post-mortem.

3.—*Bronchial Mucus*—(continued).

No. of Sheep.	Sex.	Age.	Date of Experiment.	Origin of Material.	Method.	Quantity.	Date of Death.	Remarks.
D. 4926 4962	Hamel "	Aged 3 years	21/1/13 21/1/13	Bronchial mucus, S.4681 "	Subcutaneous "	10 c.c. 10 c.c.	7/7/13 22/4/13	Killed for post-mortem. "
E. 2187	Ewe	—	20/10/11	Bronchial mucus, S.3378	Intrapulmonary	5 c.c.	28/1/13	Killed for post-mortem; used in experiments 3 B, 2 E.
3841	Hamel	Aged	7/3/12	{ S.3379 S.3880 S.3378 }	"	2 c.c.	14/3/12	Died, pleurisy and pericarditis.
3849	"	"	7/3/12		"	3 c.c.	10/3/12	"
1827	Ewe	3 years	27/10/12		"	3 c.c.	8/3/13	Killed for post-mortem; jagziente; used in experiments 2 B, 3 B.
4947	Hamel	"	21/1/13	"	"	5 c.c.	2/4/13	Killed for post-mortem.
3886	"	Aged	21/1/13	"	"	5 c.c.	28/3/13	Killed for post-mortem; jerky respirations; 28/1/13.
F 3322	—	—	27/10/11	Bronchial mucus, S.3378	Intravenous	9 c.c.	14/11/13	Killed for post-mortem; used in experiments 3 B, 2 B.

4.—*Lung Lesion Emulsion*.

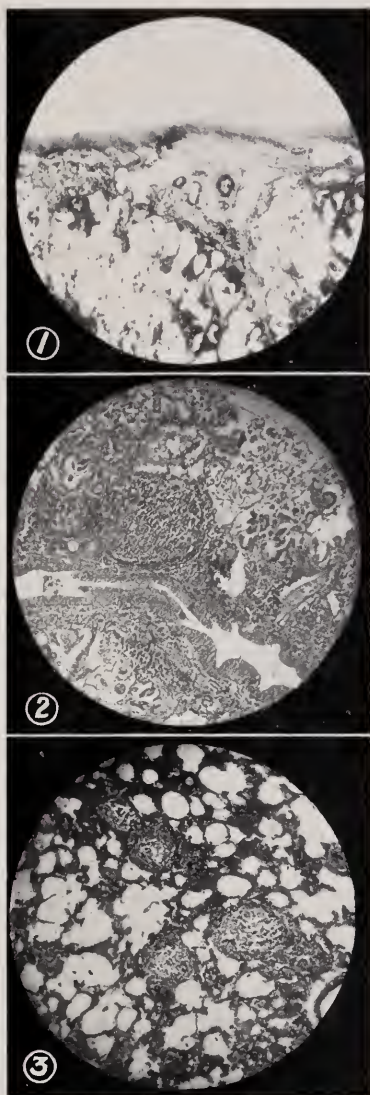
A.	Sex.	Age.	Date of Experiment.	Origin of Material.	Method.	Quantity.	Date of Death.	Remarks.
3844	Hamel	Aged	7/3/12	Lung emulsion, S.3370	Drenched	1 oz.	18/6/13	Killed for post-mortem.
3843	"	"	7/3/12	"	"	2 oz.	28/3/13	"
3843	"	"	5/6/12	"	"	?	28/3/13	"
3842	"	"	1/7/12	"	"	?	1/4/13	Killed for post-mortem; used in contact from 7/3/12.
3840	"	"	1/7/12	"	"	?	11/4/13	Killed for post-mortem; used in contact from 5/3/12 to 5/7/12.
4942	"	"	21/1/13	"	"	4 oz.	7/7/13	Killed for post-mortem.

4.—*Lung Lesion Emulsion*—(continued).

No. of Sheep.	Sex.	Age.	Date of Experiment.	Origin of Material.	Method.	Quantity.	Date of Death.	Remarks.
A.								
4955	Hamel	Aged	21/1/13	Lung emulsion.	Drenched	4 oz.	7/7/13	Killed for post-mortem.
3824	"	"	7/3/12	"	"	4 oz.	9/1/13	Died.
3838	"	"	7/3/12	"	"	20 c.c.	2/4/12	Died, poverty; used in experiment 4 C.
5054	"	"	22/5/13	"	"	300 c.c.	8/7/13	Killed for post-mortem.
B.								
2237	Ewe	Aged	27/10/11	Lung emulsion.	Intrajugular	5 c.c.	27/10/11	Died, embolism.
3224	Hamel	"	27/10/11	"	"	3 c.c.	14/2/12	Died, poverty.
4960	"	3 years	21/1/13	"	"	5 c.c.	9/4/13	Killed for post-mortem.
4961	"	"	21/1/13	"	"	5 c.c.	28/3/13	"
3823	"	"	12/2/13	"	"	10 c.c.	7/7/13	"
C.								
3846	Hamel	Aged	7/3/12	Lung emulsion.	Subcutaneous	5 c.c.	10/3/12	Died, septicaemia.
3851	"	"	7/3/12	"	"	10 c.c.	10/3/12	Died, toxæmia.
3838	"	"	7/3/12	"	"	20 c.c.	2/4/12	Died, poverty; used in experiment 4 A.
3835	"	2 years	7/3/12	"	"	10 c.c.	23/12/12	Died.
4953	"	Aged	21/1/13	"	"	10 c.c.	7/7/13	Killed for post-mortem.
4952	"	"	21/1/13	"	"	10 c.c.	7/7/13	"
5080	"	3 years	22/5/13	"	"	10 c.c.	29/5/13	Died, septic pneumonia.
5059	"	"	22/5/13	"	"	10 c.c.	8/7/13	Killed for post-mortem.
D.								
4924	Hamel	3 years	21/1/13	Lung emulsion.	Intratracheal	5 c.c.	9/4/13	Killed for post mortem.
4949	"	Aged	21/1/13	"	"	5 c.c.	7/7/13	"
4957	"	"	12/2/13	"	"	10 c.c.	7/7/13	"
3836	"	"	7/3/13	"	"	5 c.c.	23/12/12	Died.
3852	"	"	7/3/13	"	"	2-5 c.c.	—	Used in experiment 1 A.
4773	"	3 years	22/5/13	"	"	10 c.c.	6/6/13	Died, pleuro-pneumonia.
5071	"	Aged	22/5/13	"	"	10 c.c.	8/7/13	Died, poverty.

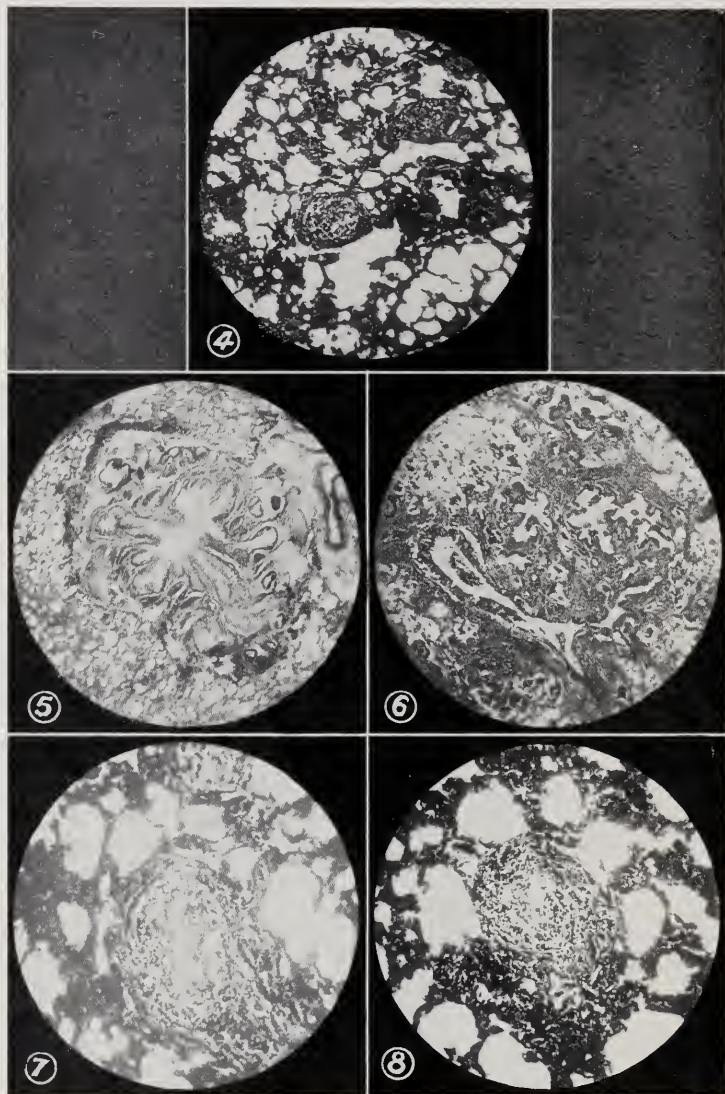
4.—*Lung Lesion Emulsion*—(continued).

No. of Sheep.	Sex.	Age.	Date of Experiment.	Origin of Material.	Method.	Quantity.	Date of Death.	Remarks.
E. 3219	Hamel	--	27/10/11	Lung emulsion, S.3878	Intrapulmonary	5 c.c.	31/10/11	Died, septic pleurisy.
3832	"	Aged	7/3/12	" S.3830	"	15 c.c.	12/3/12	Died, pleuro-pneumonia.
3825	"	"	7/3/12	" S.3379	"	5 c.c.	27/3/12	" "
3850	"	"	7/3/12	" S.3379	"	10 c.c.	17/3/12	" "
3848	"	"	7/3/12	" S.3830	"	5 c.c.	11/3/12	" "
1833	"	3 years	21/1/13	" S.3378	"	5 c.c.	7/2/13	Killed for post-mortem; used in experiments 3 B, 2 B.
4960	"	"	21/1/13	" S.4681	"	5 c.c.	3/4/13	Killed for post-mortem.
4892	"	"	21/1/13	" S.4681	"	5 c.c.	22/4/13	" "
4726	"	Aged	12/2/13	" S.3950	"	60 c.c.	15/2/13	Died, septicaemia.
3780	"	"	12/2/13	" S.3950	"	20 c.c.	18/2/13	Died, gangrenous pneumonia.
4714	"	"	12/2/13	" S.3950	"	20 c.c.	15/2/13	Died, internal haemorrhage.
4735	"	"	12/2/13	" S.3950	"	20 c.c.	18/2/13	Died, gangrenous pneumonia.
4932	"	"	12/2/13	" S.3950	"	10 c.c.	16/2/13	" "
5079	"	3 years	22/5/13	" S.5300	"	10 c.c.	29/6/13	Died, toxæmia.
5075	"	"	22/5/13	" S.5300	"	10 c.c.	1/6/13	Died, septic pneumonia.
3912	"	Aged	22/5/13	" S.5300	"	10 c.c.	26/5/13	" "
F. 4943	Hamel	3 years	12/1/13	Lung emulsion, S.3950	Intraperitoneal	10 c.c.	32/4/13	Killed for post-mortem.
G. 3845	Hamel	Aged	7/3/12	Lung emulsion, S.3830	Intramuscular	10 c.c.	23/3/12	Died, gastro-enteritis.
3834	"	"	7/3/12	" S.3830	"	20 c.c.	15/3/12	Died, toxæmia.



Jagzickte.

D. T. Mitchell.



Jagziekte.

D. T. Mitchell.

**Report on *Acokanthera Venenata*,
G. Don, from the Transvaal.**

*From the Imperial Institute of the United Kingdom, the Colonies,
and India.*

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From the Imperial Institute of the United Kingdom, the Colonies, and India.

THE attention of the Imperial Institute was first directed to this poisonous plant in 1906 by the Acting Director of Agriculture in the Transvaal, who forwarded a single small specimen of the root for identification, stating that it was suspected that this material had been used in a case of criminal poisoning among natives near Johannesburg. The root was identified at the Royal Botanic Gardens, Kew, as probably that of *Acokanthera venenata*, G. Don. In reporting this identification to the Director of Agriculture at Pretoria, the Imperial Institute submitted a short statement giving the information available regarding the constituents of other species of *Acokanthera*, and pointed out that no detailed examination of *A. venenata* had been made, though the plant was well known to be poisonous.

A small supply of leaves and twigs of the plant was subsequently received from the Director of Agriculture at Pretoria, with a letter, No. 42661 of the 11th June, 1909, which stated that in view of two or three cases of criminal and accidental poisonings having been traced to the use of the bark of this shrub it was very desirable that it should be examined chemically. This sample proved too small for detailed examination, and in December of the same year a further supply was asked for, which was received from the Director of Agriculture with letter No. 51324 of the 26th April, 1910.

This last supply of material enabled the preliminary chemical and physiological investigation of the plant to be completed, and the results of this work are summarized in the present report.

Previous Work on Acokanthera Species.

The *Acokanthera* species have long been known as poisonous plants, and they yield a well-known group of arrow poisons. The species best known in this way are *A. ouabaïo*, Cath., *A. deflersii*, Schw., *A. schimperi*, Oliv., and *A. abyssinica*, K. Schum. From these species, or from arrow poisons prepared from them, various investigators have isolated two poisonous constituents, both of which are glucosides, viz.:—

1. A crystalline glucoside, called ouabaïn or acokantherin.
2. An amorphous glucoside, variously known as abysinin, amorphous acokantherin, amorphous ouabaïn, or acokanthin.

A detailed account of these previous investigations need not be given here. They are described by Fraser & Tillie in the *Pharmaceutical Journal* for 1895, p. 76, and by Lewin in the *Berliner Klinische Wochenschrift*, 1906, p. 1583. A summary of information regarding

Acokanthera species, especially in relation to their use as arrow poisons, is given by Perrot & Vogt in *Poisons de Flèches et Poisons d'Epreuve* (Paris; Vigot Frères, 1913).

As regards *Acokanthera venenata*, Lewin examined a small quantity of the twigs of this species grown in Italy [*Arch. für. path. anat.*, Vol 134 (1893), p. 231], and isolated an amorphous glucoside, which, though toxic, did not exhibit the chemical and physiological properties of amorphous acokantherin. The only other contribution to the subject is that of Krause, who stated [*Tropenpflanzer*, Vol. 13 (1909), p. 134] that *A. venenata* contained acokantherin and abyssinin, but gave no further details of his work.

Chemical Examination of Acokanthera venenata at the Imperial Institute.

As all the species of *Acokanthera* hitherto examined have proved to contain either crystalline or amorphous ouabain (acokantherin) or both of these substances, it seemed reasonable to suppose that *A. venenata* would also prove to contain one or both of these products, especially as the symptoms of poisoning produced by it, so far as they had been observed, were quite similar to those recorded for the other species. The plant was therefore examined in the first instance by the methods used by previous workers. These may be described briefly as more or less complicated processes for the fractionation of an extract prepared by exhaustion of the plant with alcohol. Of all the methods tried, that used by Faust in his examination of *Acokanthera abyssinica* [*Arch. exp. Path. und Pharm.*, Vol 48 (1902), p. 272, and Vol. 49 (1903), p. 446] proved most convenient, and was finally used for working up the bulk of the material supplied. The method and the details of its application to *Acokanthera venenata* need not be described, but the results may be summarized as follows:—No alkaloid was detected in the plant, and no trace of any glucoside yielding prussic acid, such as has been found in certain other poisonous plants examined at the Imperial Institute, could be found. The plant, however, contains a considerable quantity of oxalic acid, and in addition an intensely bitter, amorphous product was isolated, which proved to be highly poisonous. All the other substances obtained were amorphous, and appeared to be physiologically inert.

As the quantity of the intensely bitter and poisonous product obtained was very small it could not be fractionated with a view to the possible isolation of crystalline acokantherin. It is therefore impossible to say whether the amorphous product is a single substance or a mixture. It possesses all the characters of crude acokantherin as described by Faust, and is probably identical with this substance or very closely related to it. This conclusion is amply confirmed by the results of its physiological examination recorded below.

Physiological Action of the Bitter Substance.

The investigation of the physiological action of this material was kindly undertaken for the Imperial Institute by Dr. J. H. Burn, of the Wellcome Physiological Laboratories, who has supplied the following report on his work. As Dr. Burn's results are of great scientific and practical interest they are given in detail:—

The examination of the action of a solution of the bitter substance corresponding to a 1 per cent. infusion of the plant in water shows that

it contains principles extremely like those occurring in *Digitalis* (fox-glove) leaves; this, of course, was to be expected from the known properties of the allied species *Acokanthera ouabaïo*.

Observations on the Intact Animal.

(1) *Frog*.—When the minimal lethal dose (about 0.5 mgm. of the bitter substance for a 25-gramme frog) is injected into the ventral lymph sac, after about an hour all reflexes diminish, and in three hours the frog is dead. The ventricle is then found to be tightly systolic.

Determination of Lethal Dose.—The preparation used contained one part of the bitter substance in 10 c.c. of solution. The doses are expressed in terms of the bitter substance.

Weight of Frog in grammes.	Dose.	Result.
24	1 mgm.	Died in 3 hours.
24	1 "	" 3 "
21	0.8 "	" 2 "
21	0.8 "	" 2 "
20	0.6 "	" 2 "
20	0.6 "	" 2 "
24	0.5 "	" 3 "
24	0.5 "	" 3 "
22	0.4 "	Lived.
22	0.4 "	"
22	0.3 "	"
22	0.3 "	"

That is to say the minimal lethal dose is 0.5 mgm. of the bitter substance for a 25-gramme frog. As the solution contains one part of bitter substance in 10 c.c., and each c.c. of the solution represents 100 grammes of the plant, then 0.5 mgm. of the bitter substance corresponds to 0.5 gramme of the plant.

An infusion of *Digitalis* leaves prepared by heating 2 grammes of leaves with 30 c.c. of saline solution on the water bath for half an hour in a flask with a reflux condenser gave a minimal lethal dose corresponding to 0.05 gramme of leaves. This was not a particularly active batch of leaves. It would therefore appear that, in so far as the methods of extraction are comparable, the *Acokanthera venenata* plant contained one-tenth of the amount of active principles which a moderate sample of *Digitalis* leaves possessed.

(2) *Guinea-Pig*.—When the minimal lethal dose was injected subcutaneously, after half an hour the animal began to give sharp convulsive coughs owing to the action of the principle on the respiratory centre. These soon became acute and the convulsions spread to the whole musculature of the body. Micturition and salivation took place. Death followed quickly, occurring forty minutes after the injection. Post-mortem examination showed fairly normal lungs, with a widely dilated heart. In the case of death from the minimal lethal dose, the cause of death was paralysis of the respiratory centre. Only with much higher doses was the heart found to be tightly systolic.

The minimal lethal dose was 3 mgm. of bitter substance for a 300-gramme guinea-pig.

Action on the Heart.

The resemblance of the principles of *Acokanthera venenata* to those of *Digitalis* comes out in all its detail when the preparation is examined on the isolated perfused mammalian heart. The accompanying tracing (fig. 1) shows the effect on the heart of a cat of perfusing Locke's solution containing 1 part of the bitter substance in 10,000.

Two minutes after perfusion of the solution began the "therapeutic" stage of the action was clearly seen, where the amplitude is increased and the frequency slowed. After $7\frac{1}{2}$ minutes there

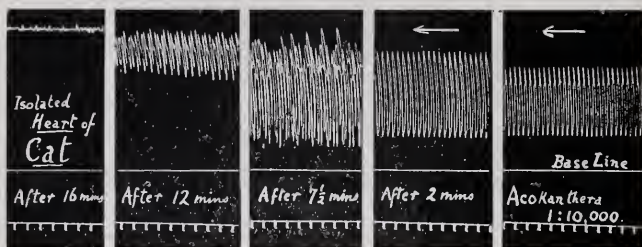


Fig. 1.

was a well-marked period of "Cushny's waves," and after 16 minutes complete quiescence in systole. During the perfusion the coronary flow gradually diminished to one-half its initial rate and then again increased slightly.

Action on the Blood Pressure.

When injected intravenously into a cat, either under ether anaesthesia or after destruction of the brain and subsequent removal of the anaesthetic, the bitter substance is seen to have a marked pressor action. A tracing (fig. 2) is attached of a cat whose brain had been destroyed, and to which a quantity of solution corresponding to 5 mgm. of the bitter substance was given intravenously. After causing a large

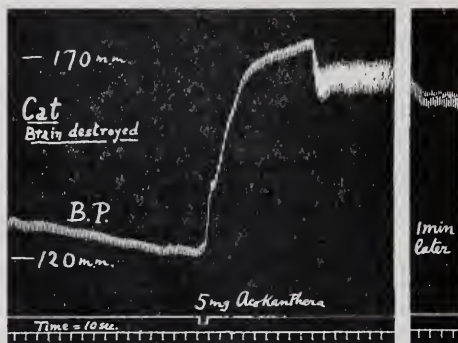


Fig. 2.

rise in the blood pressure the substance affected the heart so that this became delirious. This condition passed away in about one minute,

and the blood pressure gradually began to fall. That this rise in blood pressure is due to a definite vaso-constriction is shown by the fact that the lever attached to an intestinal plethysmograph falls when the blood pressure rises. A tracing showing this is also given (fig. 3). In this experiment the cat was under ether anaesthesia.

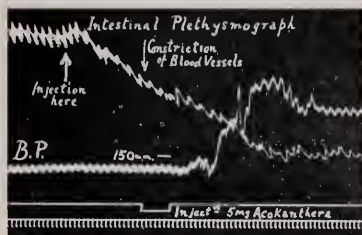


Fig. 3.

Such a vaso-constriction might be caused by a stimulus, either (a) to the sympathetic ganglia, or (b) to the nerve-endings of sympathetic nerves in the blood vessels walls, or (c) to the muscles of the blood vessels themselves.

The actual site of the application of the stimulus appears in fact to be the muscles of the blood vessels, as the rise can still be elicited after complete paralysis of the vaso-constrictor nerve-endings of the sympathetic system with ergotoxine. This is also shown in an accompanying tracing (fig. 4).

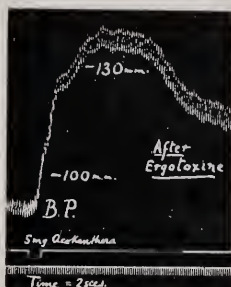


Fig. 4.

Action on Plain Muscle in General.

The action on the blood vessels would seem to be part of a general action on plain muscle. Thus a dose corresponding to 0.5 mgm. of the bitter substance put into a 50 c.c. bath of oxygenated ringer at 37° produces a large contraction of an isolated uterus of a virgin guinea-pig (see fig. 5).

Action on the Kidney.

A record of the flow or urine from the ureters into the bladder during the intravenous injection of 5 mgm. of the bitter substance showed that corresponding with the initial rise of pressure the flow decreased, but that soon there was a slight but not very considerable

increase in the rate of kidney secretion. This diuretic effect was no doubt considerably masked by the high blood pressure and the accompanying constriction in the renal arteries.

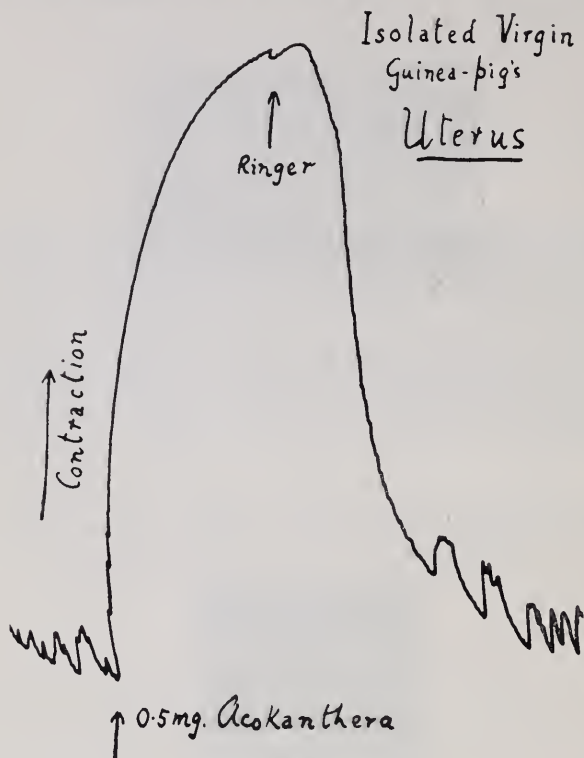


Fig. 5.

SUMMARY.

The action of *Acokanthera venenata* is therefore in every way like that of *Digitalis*. It has a primary slowing and strengthening effect on the heart which quickly passes into a toxic effect, leaving the heart quiescent in systole. It has a pressor effect when injected intravenously, due to an action on the plain muscle of the arterioles. It appears to have a contractile effect on all plain muscle. It has also a slight diuretic action.

GENERAL REMARKS.

The chief interest of the results of an investigation of this kind lies primarily in the possibility of using them in the detection of the poison in cases of accidental or criminal poisoning. The results of the chemical examination recorded in the earlier portion of this report show that the poisonous substance is so ill-defined that a chemical examination would be of little value in such cases, and reliance would have to be placed chiefly on observation of the character of the toxic

symptoms. From this point of view Dr. Burn's record of the principal physiological effects of the poison are of special interest.

The fact that *Acokanthera venenata* may also be of medicinal value as a substitute for Digitalis should not be overlooked, although it is not probable that it will be of practical value in medicine, since it is only one-tenth as active as Digitalis (foxglove), and moreover offers no advantages over Digitalis, which is obtained cheaply almost everywhere and is universally recognized as a trustworthy therapeutic agent.

7th January, 1915.

On the Transmission of *Haemoproteus columbae*.

By

Dr. RICHARD GONDER.

(Mitglied des Georg-Speyerhauses, Frankfort a/M, formerly of the
Onderstepoort Laboratories for Veterinary Research, Union of
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AT the International Congress in London in 1913, I took the opportunity, afforded in the course of a discussion following an address by v. Wasilkewsy on the blood parasites of birds, of briefly reporting upon my own investigations concerning the natural transmission of *Haemoproteus columbae*.

The discussions of that meeting have since been reported in the form of congress transactions, but since the publication includes only a condensed version of many of the papers read, and is in any case not universally accessible, I consider it advisable to publish a more detailed account of my own work treated from a somewhat more comprehensive zoological point of view.

The experiments here recorded were commenced in 1910 in the Transvaal at the Onderstepoort Laboratories for Veterinary Research (Department of Agriculture, Union of South Africa), and had for their object the verification and extension of the findings of Beaurepaire Aragao and of Sergent concerning the transmission and development of *Haemoproteus columbae* in the pigeon and in the insect carrier.

As already known, Edm. and Et. Sergent were the first to succeed in connecting *Lynchia maura* with the transmission of *Haemoproteus columbae*, and in establishing an incubation period of approximately twenty-eight to twenty-nine days in the avian host. They also showed that an infection in the lynchia was not inherited by the succeeding generation of flies, an observation which was subsequently confirmed by Beaurepaire Aragao. Aragao worked with another variety of lynchia, *Lynchia bruna* or "livicolor," and recorded an incubation period of twenty to thirty days.

Like the brothers Sergent, Aragao could only establish the development of *haemoproteus* in the lynchia up to the ookinete stage. On the other hand he found that a complete cycle of development occurred in the pigeon, the site of development being the lungs.

He infected pigeons with emulsions of stomach and intestinal contents of lynchiae, and succeeded in transmitting the infection with a shorter incubation period. Sergent has more recently done the same.

I have been able to confirm the observations of Aragao and the brothers Sergent in all respects, but, in addition, I have found that not only the lungs but also the liver may frequently become infected with large cysts. With the bite of the lynchia, the ookinetes, which lose their pigment in the stomach of the fly, are introduced into the blood-stream of the pigeons. Here they are taken up by the leucocytes, carried round to the lungs or liver, and there retained. The subsequent development of the parasite then proceeds within the enlarged

leucocytes, with the formation of cysts in which the whole schizogonous multiplication may be accepted as taking place. (Compare Bd. 12, Archiv. f. Protistenk 1903, pp. 154-167.)

The observations on the differentiation of the nucleus and the protoplasm in the cysts are interesting. I have also been able to confirm the fact that in cysts of the same age some show a more compact protoplasm staining intensively with Giemsa, with small regularly formed nuclei, while others exhibit a protoplasm of more open structure and less compact nuclei and, as a whole, take the Giemsa stain less deeply.

I should also like to draw attention here to the fact that this differentiation also occurs in the cysts of *Haemoproteus oryzivora* from the rice bird (paddy bird). In the schizogonous development of this blood parasite in the brain, liver, and spleen, two distinct types can always be made out. This difference can probably be attributed to an earlier sexual differentiation into male and female forms. The distinction lies in the degree of compactness or looseness in the structure of the nucleus and protoplasm. When the bursting of the fully charged cyst and the emerging of the small pigmentless gametocytes take place, no distinction can be discerned between the young gametocytes themselves. Only after further development in the blood cells does the distinct difference between macrogametocyte and microgametocyte appear.

Since the observations of the Sergents and of Aragao that the development of *Haemoproteus columbae* in the lynchia only proceeds as far as the ookinete stage must be regarded as unusual when compared with the life-cycle of other blood parasites, it was considered worth while to once more follow out the infection in the fly. So far, we know of no blood parasite in which there is alternation of generations in different hosts and yet in which the development in the carrier proceeds only as far as the ookinete. At most, the case of *Theileria parva*, the causal organism of East Coast fever, might be cited as analogy. In the organs of the bovine this parasite also undergoes schizogonous development right through to the gametocyte stage, in which form it attacks the blood-cells. In the transmitting tick the formation of small ookinetes occurs, and these remain until the moulting of the tick. After moulting, blood is again taken up by the tick, with concomitant infection of the attacked host. The protozoan forms previously described by me in the glands and alimentary tract of the tick after moulting, and at the time regarded as developmental stages in the life-history of *Theileria parva*, I now regard as unrelated to that parasite. I have subsequently observed similar forms in non-infected ticks on re-examination of my earlier preparations. It is therefore possible that the ticks also infect only with the small ookinetes.

In order to study the development of *Haemoproteus columbae* in the lynchia, I at first used only newly hatched flies, which, as shown both by the Sergents and by Aragao, are non-infective. The flies, identified by the Pretoria Museum as *Lynchia olfersia capensis*, were obtained from pigeons in the Laboratory dove-cote. All the pigeons of the cote were infected, and every lynchia fly taken directly from the pigeons, or caught from those free flies flying around, was found to be infected. On this account it was always possible to use unambiguous material to which no objection could be raised.

The experimental pigeons were brought from Hamburg, and, as microscopical examination had shown, contained no parasites in their blood. As a precautionary measure they were isolated in a room sealed with gauze, and so kept for a month after arrival. They were then re-examined to control a possible infection which might easily have occurred during railway transport in Africa. The fears, however, proved groundless, and not a single bird was found infected. Each pigeon was finally retested before being used for individual experiment.

Since it was found that lynchia, which had once infected a healthy pigeon, were no longer capable of infecting other healthy pigeons, it was a simple matter to obtain reliable uninfected flies. Such flies, as soon as they had cleaned themselves from infection, were maintained exclusively on healthy pigeons, and the pupae duly collected. The newly hatched flies were also reared on healthy pigeons. These pigeons—maintained for the rearing of new flies—remained free from infection during half a year. Occasionally a bird from this batch was removed for artificial infection or infected as control for immunity against infective flies. I have never been able to detect an immunity in pigeons.

EXPERIMENTS.

First Experiment.—Forty pigeon flies caught in the dove-cote were placed on a healthy pigeon (1) in a cage closed in with gauze. After twenty-four hours they were examined, and those which had not sucked blood were then transferred to a second healthy bird (2). The remainder were placed on a third healthy pigeon (3).

Pigeon (1) showed the first appearance of parasites in the blood twenty-six days after infection by the flies, and pigeon (2) after twenty-eight days. Pigeon (3) remained free from parasites. The lynchiae, which had been successfully kept alive on the healthy pigeon for over a month, were *no longer infective*. They were "cleaned" from infectivity.

Since pigeon (2) became infected, it is also obvious from this experiment that the lynchiae could retain their infectivity for one day. As the next experiment shows, a repetition of the trial along similar lines gave the same results.

Second Experiment.—Twenty-three freshly hatched flies were placed upon a well infected pigeon and left there for twenty-four hours. Those found fully engorged with blood were then transferred to a healthy pigeon (4), which, after twenty-seven days, duly showed parasites. Those flies which appeared to have sucked no blood were left upon the original infected bird, examined the following day, and transferred to healthy pigeon (5). Infection followed and parasites appeared on the twenty-ninth day.

On attempting to keep lynchiae alive without blood, it was found that they usually only survived for two days, whether infected or not. Some, however, lived to the third day, and a few infected flies surviving for this period were microscopically examined. It appeared that the ookinetes were still alive, a conclusion confirmed by an experiment in which the lynchiae previously used to infect pigeon (5) were then used to successfully infect another healthy pigeon (6). Between the two tests an interval of over forty-eight hours had elapsed, during which time the lynchiae retained their original infection—the successful infection of bird (6) being explained on the ground that some of the

infected flies must have refrained from sucking blood from pigeon (5) and had therefore escaped cleansing.

Third Experiment.—Newly hatched lynchiae which had lodged on well infected pigeons for only four to five hours, but which during this time had sucked fresh blood, were placed upon a healthy pigeon (7). Two days later the flies were transferred to another healthy bird (8). Pigeon (7) showed itself infected after the usual incubation period of twenty-nine days. No. (8) remained free from parasites. This experiment was repeated several times, in each case with the same results.

From this test it is apparent that flies which have once infected themselves can only once infect a healthy bird. As soon as they have taken up healthy blood, further infectivity can no longer be detected. One can therefore speak of a "cleansing" of the lynchia just as one can speak of the cleansing of the infective tick in the case of East Coast fever. The flies are incapable of retaining the infection, which probably for this reason cannot be transmitted through the eggs and the pupae.

Fourth Experiment.—Lynchiae, which had been once infected, but which had undergone cleansing as indicated in the preceding experiment, were once more placed upon infected pigeons. After twenty-four hours they were again examined to ensure that blood had been absorbed, and were then transferred to healthy pigeons. After twenty-three to thirty days these birds were found to have been infected exactly as they would have been by flies infected for the first time. The lynchiae, therefore, acquire no immunity.

Fifth Experiment.—Newly hatched flies were maintained exclusively on infected birds. In a parallel experiment flies caught in the dove-cote were treated in the same way. From each of these a few were occasionally removed and transferred to healthy pigeons. The lynchiae were found to be invariably infective and ookinetes were regularly detected in their stomachs.

From this experiment, as well as from that immediately preceding, it is clear that the infectivity of the fly depends solely upon the blood taken up. The tests have been repeated on several occasions. It may therefore be stated that an infected lynchia loses its infectivity when it sucks the blood of a non-infected pigeon, and that although it loses its original infection, even when it sucks the blood of an infected bird, it derives a fresh infectivity in the process.

Microscopical investigation of the stomach and intestinal contents of infected flies revealed a development of gametocytes to gametes, a fertilization and formation of ookinetes. The microgametocytes are very rapidly rounded off as soon as they reach the stomach of the fly, and the formation of microgametes occurs immediately after. The same thing also happens with the macrogametocytes. I have observed exactly the same time of development of the ookinetes in the moist chamber. After one to two hours the ookinetes had already developed. Aragao, in his experiments, observed the formation of ookinetes from two to three hours after blood absorption.

The ookinetes take the shape of a gregarine, and their movements are also like those of gregarious. By sub-division they lose their pigment, and through a second partition once again lose part of their protoplasm, so that the final ookinete is comparatively small.

No further development occurs within the lynchia. It is the small ookinete which reaches the blood of the pigeon during the bite of the fly, and which is taken up by the leucocytes. By the leucocyte it is in turn carried to the lungs or the liver of the avian host, in which organs cyst formation takes place. The cysts gradually increase in size by schizogonous multiplication and finally burst. The young gametocytes are so liberated and, while still in the organs, attack the red blood cells. The young parasites then grow exclusively in the blood, developing their sexual individuality as micro- and macro-gametocytes.

Aragao attempted to transmit infection from infected to healthy pigeons by blood inoculation, but without success. I have repeated the attempt, but also failed in all cases except one. In the one successful attempt it was found on examination that the blood used for injection already contained microgametes, macrogametes, and a few ookinetes. When perfectly fresh blood was used transmission invariably failed.

It was clear from all the experiments already described that infective lynchia can only infect healthy pigeons by means of the ookinetes. It was therefore considered of interest to attempt transmission with ookinetes cultivated in the moist chamber. For the purpose of obtaining these the blood of infected pigeons was mixed in dishes or in test-tubes with two-thirds physiological salt solution and allowed to stand for a few hours. Small samples were then removed—every five minutes at first, but subsequently every half hour—and examined microscopically in order to follow the formation of ookinetes. As mentioned above, the formation of ookinetes proceeds in the moist chamber exactly as it does in the fly, except that the subsequent segmentation of the protoplasm is rarely seen.

Pigeons were then infected with the material under consideration, and infection was found to occur with the usual incubation period of twenty-three to thirty days. On repetition of this experiment the same results were always obtained.

It may therefore be regarded as experimentally established that infection can be artificially accomplished as soon as the ookinete stage is reached, and that further development is quite unnecessary. The formation of the ookinete is, however, absolutely essential.

I have also succeeded in transmitting infection from pigeon to pigeon by using organ material from freshly infected birds. The lungs of pigeons killed from fifteen to nineteen days after infection were removed, reduced to a coarse emulsion, and injected into healthy birds. Infection followed and was usually, although not invariably, characterized by a short incubation period. The infection was generally weak, and the longest period of incubation was nineteen days. In some cases a lung puncture was made with a fine needle before fifteen days or after nineteen days from the date of initial infection in order to follow the course and extent of parasitic development.

Direct transmission such as this may be explained on the supposition that the inoculated cysts are capable of continued development in the new bird. Pigeons can also be directly infected with cysts in advanced developmental stages, and with small pigmentless gametocytes which are to be found in the free state in the lungs shortly after swarming. In such cases the incubation period only amounts to a few days (about six), and the infection is extremely weak.

Analagous instances also occur in the East Coast fever of cattle. An infection can here never be obtained by blood inoculation whatever be the stage of the disease. On the other hand, an infection with organ material is possible, since the schizogonous development takes place within the organs and is capable of simple continuation in sound cattle right up to the stage at which the gametocytes finally appear in the blood.

SUMMARY.

The infectivity of the parasite *Haemoproteus columbae* is not hereditary in the fly-carrier. The parasite develops in the fly, *Lynchia capensis* (*olfersia*), only to the ookinete, which, through a double protoplasmic segmentation, loses all its pigment and a part of its protoplasm. So long as the ookinetes are present in the stomach of the fly transmission of the parasite by the fly is possible.

The flies "clean" themselves from an infection whenever they engorge on the blood of a healthy pigeon, but do not become immune against reinfection.

A cleansing of the flies cannot take place so long as they feed upon infected pigeons.

Artificial transmission from infected pigeons to healthy pigeons cannot be brought about by ordinary blood inoculation, but is easily effected by the injection of ookinetes cultivated in the moist chamber.

Direct transmission is also possible if lung material be used.

